

SOIL SCIENCE

Editor-in-Chief
JACOB G. LIPMAN

Associate Editor
HERMINIE BROEDEL KITCHEN

Contents

- The Microflora of Leached Alkali Soils: I. Synthetic Alkali Soil. J. DUDLEY
GREAVES 341
- Composition and Nitrification Studies on *Crotalaria Striata*. W. A. LEUKEL,
R. M. BARNETTE AND J. B. HESTER 347
- The Laws of Soil Colloidal Behavior: II. Cataphoresis, Flocculation, and
Dispersion. SANTE MATTSON 373

PUBLISHED MONTHLY
THE WILLIAMS & WILKINS COMPANY
MT. ROYAL AND GUILFORD AVENUES
BALTIMORE, MARYLAND, U. S. A.

Made in United States of America

SOIL SCIENCE



FOUNDED BY
RUTGERS COLLEGE
NEW BRUNSWICK, N. J.

EDITORIAL BOARD

- | | |
|--|---|
| DR. F. J. ALWAY
University of Minnesota, St. Paul, Minn. | DR. T. L. LYON
Cornell University, Ithaca, N. Y. |
| PROF. K. ASO
Imperial University, Tokyo, Japan | DR. M. M. MCCOOL
Michigan State College, East Lansing, Mich. |
| PROF. C. BARTHEL
Central Agricultural Experiment Station, Experimentalfältet, Sweden | DR. W. H. MACINTIRE
University of Tennessee, Knoxville, Tenn. |
| PROF. A. W. BLAIR
Rutgers University, New Brunswick, N. J. | DR. E. A. MITSCHERLICH
University of Königsberg, Prussia |
| DR. P. E. BROWN
Iowa State College of Agriculture, Ames, Iowa | PROF. C. A. MOOERS
University of Tennessee, Knoxville, Tenn. |
| PROF. DR. ALBERT DEMOLON
Ministry of Agriculture, Paris, France | DR. SVEN ODÉN
Central Agricultural Experiment Station, Experimentalfältet, Sweden |
| DR. H. J. CONN
New York State Experiment Station, Geneva, N. Y. | DR. THEO. REMY
Institut für Boden- und Pflanzenbaulehre, Bonn a. Rh. |
| DR. E. B. FRED
University of Wisconsin, Madison, Wis. | PROF. G. ROSSI
Royal Agricultural High School in Portici, Naples, Italy |
| DR. J. E. GREAVES
Utah Agricultural College, Logan, Utah | DR. E. J. RUSSELL
Rothamsted Experimental Station, Harpenden, England |
| DIRECTOR ACH. GREGOIRE
Agricultural Experiment Station, Gembloux, Belgium | DR. O. SCHREINER
U. S. Department of Agriculture, Washington, D. C. |
| DR. R. GREIG-SMITH
Linnean Society, Sydney, New South Wales | DR. ALEXIUS A. J. DE'SIGMOND
Royal Hungarian Joseph University of Technical Sciences, Budapest, Hungary |
| DR. B. L. HARTWELL
Rhode Island Experiment Station, Kingston, R. I. | DR. J. STOKLASA
Technical High School, Prague, Czechoslovakia |
| DR. D. J. HISSINK
Agricultural Experiment Station, Groningen, Holland | PROF. CHAS. E. THORNE
Ohio Experiment Station, Wooster, Ohio |
| DR. C. B. LIPMAN
University of California, Berkeley, Calif. | PROF. N. M. TULAIOV
Agricultural Experiment Station, Saratov, Russia |
| DR. BURTON E. LIVINGSTON
Johns Hopkins University, Baltimore, Md. | DR. S. A. WAKSMAN
Rutgers University, New Brunswick, N. J. |
| DR. F. LÖHNIS
University of Leipzig, Germany | DR. F. WEIS
Royal Agricultural and Veterinary College, Copenhagen, Denmark |
| | PROF. S. WINOGRADSKY
Pasteur Institute, Paris, France |

THE MICROFLORA OF LEACHED ALKALI SOILS: I. SYNTHETIC ALKALI SOIL

J. DUDLEY GREAVES

Utah Agricultural Experiment Station¹

Received for publication April 29, 1929

A vast amount of work has been done on the effect of "alkali salts" upon the physical, chemical, and biological properties of soils. The biological work has dealt primarily with the chemical processes occurring in the soil, and little consideration has been given to the specific microorganisms which occur and function in such soils. It is generally known that actual qualitative and quantitative differences exist in the vegetation of "alkali" and "non-alkali soils," and it is not unreasonable to expect a similar variation in the microflora. Moreover, the unproductivity of recently leached alkali soil is probably due in a measure to the absence of the necessary microorganisms. For these reasons a rather comprehensive study has been made of the morphological and physiological properties of the microorganisms occurring in recently leached "alkali soils."

Three different soils were studied: (a) A synthetic "alkali soil," (b) a natural-occurring "alkali soil" rich in chlorides, and (c) a natural-occurring "alkali soil" rich in sulfates. This paper deals with the synthetic soil; the others will be considered in subsequent papers.

PREPARATION OF SOIL

The synthetic alkali soil was prepared by adding 0.66 per cent each of sodium chloride, sodium sulfate, and sodium carbonate to a naturally productive sandy loam. The moisture was made up to 20 per cent and maintained at this level for two months for the soil reactions to reach equilibrium. It was then leached for a period of 199 days, 240 liters of tap water passing through it (7). Analyses of the leachings showed that 91.3 per cent of the sodium chloride, 97.7 per cent of the sodium sulfate, and 43 per cent of the sodium carbonate were recovered. The leaching had increased the bacteria 237 per cent; the ammonifying powers, 336 per cent; the nitrifying powers, 3350 per cent; and the nitrogen-fixing powers, 357 per cent.

The pots were sampled and then seeded to crimson clover, and the plants and roots harvested at time of full bloom, after which the soil was again sampled

¹ Contribution from the department of bacteriology and chemistry. Publication authorized by the director, March 13, 1929.

It is with pleasure that acknowledgment is made to H. C. Pulley and C. E. ZoBell for making some of the chemical tests.

and analyzed. It was found that the ammonifying powers of the leached soil had increased 4.7 per cent and the nitrifying powers, 40 per cent; the nitrogen-fixing powers had decreased 66 per cent, whereas the total number of colonies developing on agar had increased 31 per cent. The pots were then seeded to barley, which was harvested at the time of maturity, after which they were resampled and analyzed. The total number of colonies had decreased 28 per cent, the ammonifying powers had increased 9 per cent, the nitrifying powers had increased 34 per cent, and the nitrogen-fixing powers had decreased 11 per cent. A third crop of barley was grown on the soil, after which the samples used in this work were taken (6, 7).

Method of isolation. The total number of colonies developing on nutrient agar (8), synthetic agar (8), and Ashby agar (4) was determined as follows: The samples were air-dried and ground to pass through an 80-mesh sieve. Five grams of soil were added to 100 cc. of sterile water and shaken for five minutes. Further dilutions were made and platings made from dilutions of 1 to 20,000 and 1 to 200,000. The average from six determinations was:

Nutrient agar	4,505,000
Synthetic agar	4,567,000
Ashby agar	2,808,000

There is a very close agreement between the number of colonies developing upon nutrient and synthetic agar, but the Ashby agar yielded only 61.5 per cent as many colonies as did the synthetic agar. For the pure-culture study, colonies were fished out of the Ashby agar plates and inoculated on Ashby agar slants, the Ashby media being selected for the following reasons: (a) Probably less work has been done on the colonies developing on this media than on the other two; (b) this soil was outstanding in its nitrogen-fixing powers; (c) many different organisms were evidently developing on this media, and the likelihood of getting the nitrogen fixers was greater than if the other media were used. The different colony types developing on Ashby agar were obtained in pure cultures by repeated dilutions, plating, and microscopic examination. The stock cultures were kept on soil extract Ashby agar. The soil extract was prepared by extracting 2 gm. of soil with 100 cc. of distilled water.

Ammonification. The ammonifying powers of the organisms were determined by inoculating each organism into 100 cc. of sterile 1 per cent peptone solution made from tap water and bacto-peptone. This was incubated for four days at 28°C. and then analyzed for ammonia by the Kjeldahl method, magnesium oxide being used as the base.

Urea decomposition was determined by inoculating 100-cc. portions of 1 per cent urea solution with the various organisms. These were incubated for four days at 28° and the ammonia determined.

Cellulose decomposition. All organisms were plated on cellulose agar (2). The degree of cellulose decomposition was determined by the area of the clear zone around the colonies. No. 7 failed to grow, and none of the organisms were able to decompose cellulose.

Nitrogen fixation. One-hundred-gram portions of finely ground garden soil were weighed into Erlenmeyer flasks and the moisture made up to 20 per cent with distilled water. These samples were autoclaved for two and one-half hours at 120°C., after which 10 cc. of distilled water, containing 1.5 gm. of mannitol, were added to each flask. The flasks were then inoculated in duplicate with each organism. Sterile checks were run. The samples were incubated for five weeks at 28°C., the moisture content being maintained at 26 per cent. At the end of three weeks of incubation an additional gram of mannitol was added, as a test had shown the initial amount to have been all used. At the end of five weeks the samples were dried at 120°C., ground, and the total nitrogen determined by the official Gunning method. Each culture was studied in detail, both morphologically and physiologically, according to the pure culture methods, but only a brief description of each organism is given. The ammonifying, urea-decomposing, and nitrogen-fixing powers are stated in milligrams as produced under the specifically described method. The organisms are probably not listed in Bergey's Manual (2) and are probably new species or varieties. They represent the microflora of this soil which will grow on nitrogen-free media and give a fair idea of the kind of organisms which develop on the Ashby media.

1A. Non-motile, gram-negative cocci 0.5 to 0.7 μ in diameter, occurring singly and in clusters. Grows well on all the ordinary laboratory media. Slowly liquefies gelatin. Abundant light tan growth on potato. Peptonizes milk with alkaline reaction. Produces indole. Does not reduce nitrates. Hydrolyzes starch but does not ferment other carbohydrates. Facultative aerobic. Produces 6.29 mgm. of ammonia and fixes 1.4 mgm. of nitrogen in soil.

1B. Non-motile, gram-negative, sporulating, rods 0.8 to 1.2 by 1.2 to 1.8 μ , occurring singly in pairs and clusters. Grows well on all ordinary laboratory media. Abundant tan growth on potato. Rapid gelatin liquefier. Slow peptonization of milk with alkaline reaction. Does not form indole nor reduce nitrates. Starch hydrolyzed but no acid produced on glucose, lactose, or sucrose. Aerobic. Produces 7.65 mgm. of ammonia and fixes 2.8 mgm. of nitrogen.

4. Gram-negative cocci 0.7 to 1.2 μ , occurring in clusters and pairs. Slow growth on ordinary laboratory media. Liquefies gelatin. Abundant tan growth on potato. Slow peptonization of milk with alkaline reactions. Does not produce indole nor reduce nitrates. Hydrolyzes starch but does not produce acid on glucose, sucrose, or lactose. Aerobic. Produces 6.46 mgm. of ammonia and fixes 4.2 mgm. of nitrogen in soil.

6B. Gram-positive rods 0.9 to 1.2 by 1.9 to 3.3 μ , occurring in chains. Motile by means of mono-trichious flagellum. Terminal spherical spores 0.5 to 0.7 μ . Grows readily on all ordinary laboratory media except potato. Abundant peach-colored growth on agar. Liquefies gelatin rapidly. Hydrolyzes starch. Produces indole. Reduces nitrates. Produces no acid on glucose, lactose, or sucrose. Aerobic. Produces 6.12 mgm. of ammonia and fixes 5.6 mgm. of nitrogen in soil.

6C. Gram-positive, motile rods 0.8 to 0.9 by 2 to 2.3 μ , occurring singly and in pairs. Central spores 1.0 to 1.8 μ . Rapid growth on all ordinary media except potato. Slowly liquefies gelatin. Does not produce indole nor reduce nitrates. Hydrolyzes starch. Produces acid on dextrose but not on lactose or sucrose. Aerobic. Produces 5.44 mgm. of ammonia and fixes 2.8 mgm. of nitrogen in soil.

7. Mold: Mycelium septate, straight with little branching. Conidia formed as in *Fusarium*; aerial mycelium formed, abundant growth on all ordinary laboratory media. Liquefies

gelatin. Hydrolyzes starch. Reduces nitrates to nitrites. Produces acid on glucose but not on lactose or sucrose. Aerobic. Produces 17 mgm. of ammonia from urea and 6.33 mgm. from peptone. Fixes 2.8 mgm. of nitrogen in soil.

10B. Gram-positive motile rods 0.3 to 0.6 by 1.4 to 3.6 μ . Abundant growth on all ordinary laboratory media. Rapidly liquefies gelatin. Abundant tan growth on potato. Indole formed. Reduces nitrates with the production of gas. Starch hydrolyzed. Aerobic. Produces 276 mgm. of ammonia from urea and 7.48 mgm. of ammonia from peptone. Fixes 1.4 mgm. of nitrogen in soil.

11A. Gram-negative, non-motile rods 0.5 to 0.6 by 2.4 to 6 μ occurring singly and in clusters. Grows well on ordinary media, does not liquefy gelatin. Slowly peptonizes milk with alkaline reaction. Produces indole, does not reduce nitrates, does not ammonify peptone. Fixes 7.0 mgm. of nitrogen in soil.

12A. Gram-positive, non-motile, non-spore-forming rod 0.6 to 0.8 by 1.5 μ , occurring singly and in clusters. Grows rapidly on agar and potatoes, slowly on gelatin and broth. Slowly peptonizes milk with the reduction of litmus. Produces indole. Does not reduce nitrates. Hydrolyzes starch, produces no acid on glucose, lactose, or sucrose. Aerobic. Produces 3 mgm. of ammonia from urea and 9.7 mgm. of ammonia from peptone. Does not fix nitrogen.

12B. Mold: Mycelium septate, straight with little branching. No aerial mycelium formed. Chlamydospores and conidia formed. Conidia oval in shape and connected in long chains. Rapid growth on ordinary laboratory media. Rapidly peptonizes milk first with acid, later turning alkaline. Blue-green, abundant growth on potato. Nitrates not reduced, starch hydrolyzed. Aerobic. Produces 4 mgm. of ammonia from urea and 167 mgm. of ammonia from peptone, does not fix nitrogen.

15A. Gram-positive, non-motile. Micrococci 0.8 to 1.1 μ in diameter. Rapid growth on agar. Slow growth on gelatin without liquefaction, produces no growth on potato. Produces indole, reduces nitrates, hydrolyzes starch, produces acid on glucose but not on sucrose nor lactose. Produces 4.6 mgm. of ammonia from peptone and fixes 4.2 mgm. of nitrogen in soil.

16B. Gram-positive, non-spore-forming rods 0.6 to 0.7 by 1.0 to 1.7 μ , occurring singly. Grows rapidly on all ordinary laboratory media and produces light yellow pigment on agar and potato. Liquefies gelatin. Slowly peptonizes milk with change in reaction. Reduces litmus. Produces indole. Reduces nitrates with the formation of gas. Hydrolyzes starch, produces acid on glucose and sucrose but not on lactose. Aerobic. Produces 8.5 mgm. of ammonia on peptone and fixes 7 mgm. of nitrogen in soil.

16C. Gram-positive, non-motile, non-spore-forming rods 0.8 to 0.9 by 1.8 to 2.7 μ , occurring singly, in pairs, and in short chains. Slowly liquefies gelatin. Rapid growth in other laboratory media. Potato abundant light cream tan growth. Produces indole. Does not reduce nitrates, hydrolyzes starch, produces acid on glucose but not on sucrose nor lactose. Aerobic. Very slowly ammonifies peptone.

16E. Gram-positive, non-motile, non-spore-forming rods 0.5 to 0.6 by 0.7 to 0.9 μ , occurring singly and in clusters. Rapid growth on ordinary laboratory media. Creamish tan growth on potato. Liquefies gelatin. Produces indole. Does not reduce nitrates nor hydrolyze starch. Produces acid on glucose but not on sucrose or lactose. Aerobic. Slowly ammonifies peptone. Fixes 5.6 mgm. of nitrogen in soil.

17B. Gram-negative, non-motile, micrococci 0.8 to 1.0 μ . Rapid growth on agar. Slow growth without liquefaction on gelatin. No growth on potato. Hydrolyzes starch, reduces nitrates, and produces acid on glucose and lactose, but not on sucrose. Aerobic. Produces 13.8 mgm. of ammonia in peptone and fixes 5.6 mgm. of nitrogen in soil.

The morphological and physiological properties of the various organisms are summarized in table 1.

It is evident, from the results reported in table 1, that of the 17 organisms only 1 is able to ferment lactose, 3 sucrose, and 9 glucose. Not one produced gas. All but 3 liquefied gelatin. A majority of the organisms peptonized milk. The reaction in the majority of cases was alkaline.

With one exception, all organisms were aerobic, and this one was facultative anaerobic. There were 10 bacilli, 4 cocci, and 1 mold. Of the 16 cultures 6 formed spores, while only 4 were motile. Of the 16, 12 hydrolyzed starch at varying rates, 11 liquefied gelatin, 6 reduced nitrates to nitrites, 10 were

TABLE 1

Summary of principal morphological and physiological properties of organisms from leached synthetic alkali soil that will grow on nitrogen-free media

CULTURE NUMBER	NITRATE REDUCTION	GELATIN LIQUEFACTION	HYDROLYSIS OF STARCH	MILK PEPTONIZED	INDOLE PRODUCTION	LITMUS MILK REACTION	ACTION ON GLUCOSE	ACTION ON LACTOSE	ACTION ON SUCROSE	SHAPE*	MOTILITY	GRAM REACTION	CHROMOGENESIS	SPORE FORMATION	AMMONIFICATION NH ₃ PRODUCED	N ₂ FIXED PER 100 GM. SOIL
															mgm.	mgm.
1a	-	+	+	+	+	Alkaline	0	0	0	C	-	-	-	-	6.3	1.4
1b	-	+	+	+	-	Alkaline	0	0	0	B	-	-	-	+	7.7	2.8
4	-	+	+	+	-	Alkaline	0	0	0	C	-	-	-	-	6.5	4.2
6b	+	+	+	-	+	Acid	0	0	0	B	+	+	+	+	6.1	5.6
6c	-	+	+	-	-	Acid	+	0	0	B	+	+	+	+	5.4	2.8
7	+	+	-	+	-	Acid and alkaline	+	0	0	F	-	+	+	+	6.6	2.8
9a	-	+	+	+	-	Acid	+	0	+	B	+	+	-	+	9.4	0.0
10b	+	+	-	+	+	Alkaline	0	0	0	B	+	+	+	+	7.5	1.4
11a	-	-	-	+	+	Alkaline	+	0	0	B	-	-	-	-	0.7	7.0
12a	-	-	-	+	+	Alkaline	0	0	0	B	-	+	-	-	9.7	0.0
12b	-	+	+	+	-	Alkaline and acid	0	0	+	F	-	+	-	-	16.7	0.0
15a	+	-	+	-	+	Alkaline	+	0	0	C	-	+	+	-	4.6	4.2
16b	+	+	+	+	+	0	+	0	+	B	-	+	+	-	8.5	7.0
16c	-	+	+	+	+	0	+	0	0	B	-	+	+	-	0.5	
16e	-	+	-	+	+	Acid	+	0	0	B	-	+	-	-	0.2	5.6
17b	+	-	+	-	-	0	+	+	0	C	-	-	+	-	13.8	5.6

* C, cocci; B, rods; F, filamentous.

gram-positive, and 9 produced indol. None of the organisms decomposed cellulose, 5 fermented urea, all but 3 produced ammonia, and 7 fixed appreciable quantities of nitrogen.

Of the 15 organisms tested, 12 fixed nitrogen when grown in soil but up to date no appreciable nitrogen fixation has been obtained in synthetic media. The gains in nitrogen which have been obtained in soils vary from 1.4 to 7 mgm. per 100 gm. of soil. Seven of the soils showed gains of over 4 mgm. of nitrogen. All but 3 of the organisms ammonified peptone, 1 produced 16.7 mgm. Four organisms ammonified urea, 1 producing 276 mgm.

SUMMARY

A study was made of the microorganisms which developed on Ashby agar from a leached synthetic alkali soil. Sixteen organisms were obtained in pure culture. Of this number 12 fixed nitrogen. The nitrogen-fixing ability of these varied from 1.4 mgm. to 7 mgm. Seven fixed over 4 mgm. in the 5-week incubation period on sterile soil to which 2.5 per cent mannitol had been added. Of the 16, 12 produced over 5 mgm. of ammonia in 4 days on a 1 per cent peptone solution. It was found that 5 of the organisms decomposed urea, whereas not one decomposed cellulose.

REFERENCES

- (1) Association of Official Agriculture Chemists. 1920 Official and Tentative Methods of Analysis, p. 8. Assoc. Off. Agr. Chem., Wash., D. C.
- (2) BERGEY, D. H. 1923 Manual of Determinative Bacteriology. Baltimore.
- (3) FRED, E. B. 1916 Soil Bacteriology. Philadelphia.
- (4) GREAVES, J. E. 1918 Azofication. *Soil Sci.* 6: 190.
- (5) GREAVES, J. E. 1927 The influence of soluble salts and organic manures on soil nitrogen. *Proc. and Papers, 1st Internat. Congr. Soil Sci.* 3: 213-221.
- (6) GREAVES, J. E. 1927 The microflora and productivity of leached and non-leached alkali soil. *Soil Sci.* 23: 271-302.
- (7) GREAVES, J. E., HIRST, C. T., AND LUND, Y. 1923 The leaching of alkali soil. *Soil Sci.* 16: 407-426.
- (8) Society of American Bacteriologists (publishers). 1928 Pure Culture Methods.

COMPOSITION AND NITRIFICATION STUDIES ON CROTALARIA STRIATA¹

W. A. LEUKEL, R. M. BARNETTE AND J. B. HESTER²

Florida Agricultural Experiment Station

Received for publication April 29, 1929

Since the work of Krause and Kraybill (5) and others on the difference of plant composition at different growth stages has established a relationship between such plant compositions and the various plant functions, many horticultural and agronomic practices are being conducted with these findings as a basis. This variation in plant composition at different growth stages has not only been correlated with plant functions, but also, with the rate of the decomposition of plant material when incorporated with the soil. Thus an accurate analysis of plant materials to be incorporated with the soil, together with suitable observations on the biological processes which take place therein, should give a more accurate picture of the problem of the accumulation and utilization of organic matter in the soil, and lead to an adequate explanation of the numerous green manure practices.

The decomposition of plant materials in the soil has been studied from several angles. Thus, the end-products of the plant constituents have been measured. Carbon dioxide and nitrate nitrogen have been used as a measure of the decomposition of plant constituents. The accumulation of so-called humus has also been measured. Finally, the response of a crop following the incorporation of organic matter with the soil has been utilized as a measure of the decomposition of organic plant constituents in the soil. Without an accurate knowledge of the composition of the plant material incorporated, it is doubtful whether any or a combination of these various methods would be of very great value in an interpretation of the decomposition of plant material in the soil. Thus, the composition of the plant material largely controls the end-products which are obtained and the ultimate effects on plant growth and the accumulation of organic residues in the soil. The processes taking place, and consequently the end-products, naturally change with the environment of the soil; particularly in regard to oxygen supply, but the composition of the plant material must necessarily control the formation of such products.

The carbon-nitrogen ratio of soil organic matter and of plant and animal

¹ Contribution from the departments of agronomy and chemistry, Agricultural Experiment Station, University of Florida. Published with the approval of the director of the Florida Experiment Station.

² Associate agronomist and associate and assistant chemists, respectively.

materials incorporated with soil has been used to determine what might be expected in end-products from their decomposition. Although the nitrogen-carbon ratio of decomposing organic matter gives in general a fair idea of the end-products to be expected, still the exceptions show that a more detailed analysis of materials is necessary to understand the processes which take place and the ultimate end-products which may be expected from a given material. Thus, a more detailed analysis of plant materials giving the proportions of the nitrogen-bearing compounds (or forms of nitrogen) to those bearing no nitrogen will give a better conception of the processes taking place in the soil when such materials are incorporated therewith. The energy relationships may certainly be better ascertained. With these relationships better understood, the selection and utilization of many of our green manuring crops should be better realized.

Among the more recently introduced green manure plants in the southeastern states, the *Crotalaria striata* stands out as one of the most promising, especially for the light sandy soils of Florida. The general characteristics and growth habits of this plant and its value as a green manure measured by crop response as given by Stokes (7) necessitated a more detailed study of its varying composition at different growth stages and the effect of these differences in composition upon its decomposition in the soil as measured by nitrate formation and accumulation.

COMPOSITION OF CROTALARIA PLANT

Preparation of Material

The *Crotalaria* plants for composition studies were grown under greenhouse conditions in 2-gallon jars with 10 kilos of air-dried Norfolk sand kept at an optimum moisture content of 50 per cent of its water-holding capacity. Each jar contained five plants. The plants from a sufficient number of jars, generally six to eight, were dug at different growth stages as follows: when young and succulent; in an advanced vegetative growth stage; when in bloom; in seed stage; in late fall or semi-dormant condition; and prepared for analysis. The roots were separated from the tops and washed in cold running water to remove adhering soil particles. The green weights of both tops and roots were recorded. They were then cut into small pieces (one-quarter to one-eighth inch in length) and 250 gm. of each placed in wide-mouthed bottles. Enough concentrated hot 95 per cent ethyl alcohol was added to each to insure a concentration of 75 per cent. The bottles were then stoppered with corks covered with tinfoil and placed in a dry room for analysis later. Duplicate 15-gm. samples of each, tops and roots, were placed in an electric oven at 105°C. to determine the percentage of dry matter. All results were computed on a dry weight basis except the percentage of dry matter.

Methods of plant analysis

Easily hydrolyzed carbohydrates. Three-gram samples in triplicate were used for analysis and results computed on a dry weight basis. The reducing

power of the various carbohydrate extractions was determined by the Schaffer-Hartman (6) volumetric method for sugar analysis, after appropriate hydrolysis, where necessary. All carbohydrate percentages were expressed in terms of glucose.

Sugars. The alcohol extract from the preserved material, after standing for several weeks, was filtered into a beaker. The preserved material was placed upon the filter paper and washed with a volume of warm 95 per cent alcohol equal to the volume of the extract. The filtrate was then evaporated on a steam bath to a syrup. The residue was then taken up with distilled water, clarified with neutral lead acetate, and filtered through dry folded filter paper. A sufficient quantity of a mixture of nine parts of anhydrous Na_2SO_4 and one part of Na_2CO_3 was added to the filtrate to remove any excess of lead. After the solution was filtered through fine filter paper, it was made up to 500 cc. Fifty-cubic centimeter aliquots without hydrolysis were used to determine reducing sugars. A 50-cc. aliquot was diluted to 90 cc., 10 cc. of concentrate HCl (sp. gr. 1.12) added, and hydrolyzed on a sand bath for one hour. The reducing power was then determined for total sugars after the solution was neutralized with 40 per cent NaOH as for reducing sugars. The residue on the filter paper was dried in a ventilated oven at 70°C . This dried material was then ground in a Quaker mill and pulverized in a Dreef (11) mill until fine enough to pass through a 60-mesh sieve. It was then preserved in tightly stoppered bottles for analysis later. Three-gram samples of this dried material were used for carbohydrate analysis. After being redried in a Cenco vacuum oven at 60°C . the total and reducing sugars were determined on the entire residue from the 3-gm. samples. These sugars, after the removal of the alcohol extract, were then computed and added to the weights of similar sugars found in the alcoholic extract. The percentage of each sugar was then computed on the total dry material in the preserved sample.

Soluble starches and dextrins. After the sugars were extracted from the sample, 50 cc. of water was added to the residue in a beaker and allowed to stand for 12 hours. This mixture was then filtered and the residue washed until the filtrate attained a volume of 90 cc. Ten cubic centimeters concentrated HCl (sp. gr. 1.12) was added to the filtrate, which was then hydrolyzed on a sand bath under a reflux condenser for two and one-half hours. The solution was then cooled, neutralized with 40 per cent NaOH, and made up to 250 cc. Fifty-cubic centimeters aliquots were used to determine the reducing power.

Starch. The residue, after the extraction of soluble starches and dextrins, was washed into a beaker and the volume made up to 50 cc. The mixture was then heated to boiling to change the starch to a paste. The temperature was lowered to 38°C ., 10 cc. of fresh saliva added, and the mixture digested at this temperature for one-half hour. At the end of this digestion period, the mixture was again heated to boiling, filtered, and the residue thoroughly washed with hot water until the filtrate attained a volume of 90 cc. Next 10 cc. of

concentrated HCl (sp. 1.12) was added to the filtrate, which was hydrolyzed on a sand bath under a reflux condenser for two and one-half hours. The solution was then cooled, neutralized with 40 per cent NaOH, and made up to a 250 cc. volume. To determine the reducing power, 50-cc. aliquots were used.

Hemicelluloses. To remove hemicelluloses, the final residue (after removal of starches) was washed into an Erlenmeyer flask, made up to 90 cc. volume, 10 cc. of concentrated HCl (sp. 1.12) added, and hydrolyzed on a sand bath under a reflux condenser for two and one-half-hours. The mixture in the flask was then filtered and washed with hot water. The filtrate was next neutralized, clarified, delead, and made up to a 500 cc. volume. To determine the reducing power, 50 cc. aliquots were used.

Cellulose. The residue (after the extraction of hemicelluloses) was boiled in 100 cc. of 1 per cent NaOH under a reflux condenser for one-half hour. This mixture was then filtered on a Hirsch funnel with suction and washed several times with distilled water. After the water was extracted with suction, the material was exposed to chlorine gas for 20 minutes. After chlorination, the fiber was washed with sulfurous acid and then boiled in 100 cc. of 2 per cent Na_2SO_3 , filtered, washed twice with hot water, and then again removed to a beaker and chlorinated for five minutes. The washing with sulfurous acid and boiling with 2 per cent Na_2SO_3 was repeated until the acid remained practically colorless when added to the tissue. The fiber was then washed well four to six times with hot water, followed by washing with 5 per cent acetic acid. The acetic acid was washed out with hot water. The fiber was removed to an alundun crucible, washed with alcohol and finally with ether. The percentage of cellulose was determined by loss on ignition.

Lignin. A 3-gm. sample in triplicate was extracted with ether (alcohol free anhydrous) to remove fats, waxes and resins, and then transferred to a beaker. Fifteen cubic centimeters of 73 per cent H_2SO_4 was added and the mixture allowed to stand for 16 hours. The mixture was then diluted with water to a 3 per cent solution of H_2SO_4 and boiled for two hours to coagulate the residue. The mixture was then filtered through an alundun crucible and washed well with hot water. The percentage of lignin was determined by loss on ignition.

Nitrogen. The various forms of nitrogen in the roots, tops, leaves, and stems of the plants were determined on the green material and the percentage of each was computed on a dry weight basis. Fifty grams of the green material of each plant part were triturated in a large mortar with quartz sand previously washed free of foreign material. About 5 cc. of ether was added to promote plasmolysis, and water was added as required to give a proper consistency for trituration. When the material was finely ground (so as to break up the plant cells), it was extracted with distilled water through four thicknesses of cheesecloth, and the extract was then filtered through a moist paper pulp filter in a Buchner funnel with slight suction to a volume of 1900 cc. The filtrate was

then made up to a volume of 2000 cc. with distilled water drawn through the paper pulp.

Forms of extracted nitrogen. One hundred cubic centimeters of the extract was used to determine total water-extracted nitrogen by the Kjeldahl method modified to include nitrate nitrogen. Two hundred and fifty cubic centimeters aliquots were brought to boiling and the coagulable proteins precipitated with 10 per cent acetic acid. After the precipitate was filtered and washed with hot water, it was transferred with the filter paper to a Kjeldahl flask, and total nitrogen determined therein by the Kjeldahl method. Aliquots from the filtrate were used to determine amino acid and nitrate nitrogen. Amino acid nitrogen was determined by the Van Slyke method. Nitrate nitrogen was determined by the DeVarda method (1). The percentage of total extracted nitrogen minus that of the coagulable protein is here termed "water-soluble nitrogen."

Unextracted nitrogen. Unextracted nitrogen was obtained by subtracting the percentage of total water-extracted nitrogen from the percentage of total nitrogen.

Total protein nitrogen. The percentage of coagulable protein nitrogen was added to that of the unextracted nitrogen and termed total "protein nitrogen."

Total nitrogen. Total nitrogen was determined on 3-gm. samples of the originally dried material by the Kjeldahl method modified to include nitrate nitrogen.

Experimental data

The data tabulated in tables 1 and 2 show a striking variation in the percentages of dry matter, the various carbohydrate compounds, and the different forms of nitrogen or nitrogen compounds from one growth stage to another during the season. Total sugars and the easily hydrolyzable polysaccharides; namely, dextrans, starches, and hemicelluloses, are comparatively high in percentage during the early succulent growth of the plants but show a marked decrease in percentage when the plants are in a more advanced vegetative growth stage. This may be accounted for by the rapid growth of the plants and the increased demand by the plant for these materials as a result of its increased respiration and anabolic processes. These compounds appear to be more constant in percentage in the different plant parts through the successive growth stages.

The percentages of cellulose in the tops and roots and the whole plant show a gradual increase from 15.86, 13.51, and 15.50, respectively, in the early succulent growth stage to 27.37, 31.37, and 28.55 for these respective plant parts in the late fall or dormant growth stage. A comparison of the lignin in the roots, tops, and the whole plant from one growth stage to another does not show much variation in percentage, but remains more or less constant. The dry matter in the tops, roots, and the whole plant shows a gradual increase in percentage from

TABLE 2
Percentage of dry matter and different forms of nitrogen in tops, roots, and whole plant of Crotalaria striata at different stages of growth during 1926
 All percentages except dry matter calculated on a dry weight basis

STAGE OF GROWTH	DRY MATTER			WATER-EXTRACTED NITROGEN			NON-WATER-EXTRACTED NITROGEN			COAGULABLE PROTEIN NITROGEN		
	Tops	Roots	Whole plant	Tops	Roots	Whole plant	Tops	Roots	Whole plant	Tops	Roots	Whole plant
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Succulent.....	13.785	9.510	12.920	1.438	1.567	1.449	2.042	0.536	1.812	0.687	0.770	0.689
Late vegetative.....	15.311	11.430	14.443	1.282	1.072	1.238	1.521	0.461	1.339	0.528	0.447	0.512
Early bloom.....	20.700	15.450	20.047	0.747	0.997	0.770	1.484	0.203	1.361	0.274	0.357	0.281
Bloom and seed pod.....	29.900	23.887	28.839	0.782	0.425	0.712	0.824	0.625	0.784	0.436	0.379	0.423
Late seed pod or dormant.....	42.473	26.185	36.089	0.471	0.412	0.454	0.652	0.970	0.742	0.245	0.311	0.263
	TOTAL PROTEIN NITROGEN			WATER-SOLUBLE NITROGEN			TOTAL NITROGEN					
Succulent.....	2.729	1.306	2.501	0.751	0.797	0.760	3.480	2.103	3.261			
Late vegetative.....	2.049	0.908	1.851	0.754	0.625	0.726	2.403	1.533	2.577			
Early bloom.....	1.758	0.560	1.642	0.473	0.640	0.478	2.231	1.200	2.131			
Bloom and seed pod.....	1.260	1.004	1.207	0.346	0.046	0.289	1.606	1.050	1.496			
Late seed pod or dormant.....	0.897	1.281	1.005	0.226	0.101	0.191	1.123	1.382	1.196			

the early succulent stage to the late fall or dormant stage. On a quantity basis, this increase in dry matter would naturally show a marked increase in actual plant material and quantities of nitrogen and carbohydrate compounds from one growth stage to another.

The tops of the plants and the whole plant show similar variations in percentages of the different forms of nitrogen. The total nitrogen, total water-extracted nitrogen, total protein nitrogen, and unextracted nitrogen show a gradual decrease in percentage from the early succulent through the successive growth stages up to dormancy. A similar trend in percentage is shown in the roots except in the case of the unextracted, total, and total protein nitrogen, which show a slight increase in percentage in the two later growth stages (table 2).

This variation in the percentages of the various carbohydrate compounds and different forms of nitrogen or nitrogen compounds from one growth stage to another naturally creates a striking difference in the relation or ratio between these compounds at these different growth stages. The higher percentage of these various nitrogen forms in the early growth stages produces a narrow carbohydrate-nitrogen ratio, whereas a decrease in the percentage of these different forms of nitrogen compared with the higher percentages of some of the higher carbohydrates in the later growth stages produces a wide carbohydrate-nitrogen ratio during the later growth stages.

This widening in the ratio between nitrogen and carbohydrates is most pronounced when these nitrogen percentages are compared with the percentages of cellulose from one growth stage to another. A gradual decrease in these nitrogen forms and the increase in the percentage of carbohydrates makes the widening of this carbohydrate-nitrogen ratio from one stage to another very pronounced.

COMPOSITION OF *Crotalaria striata* IN RELATIONSHIP TO ITS DECOMPOSITION IN THE SOIL

A marked difference was noted in the percentages of different carbohydrate compounds and various forms of nitrogen or nitrogen compounds in the foregoing study of the *Crotalaria* plant. Likewise, a striking difference in the ratio between the carbohydrate compounds and the different forms of nitrogen or nitrogen compounds was shown. These variations in plant composition indicate that there would exist a difference in the formation and accumulation of the different end-products of decomposition when these materials are incorporated with the soil. For the purpose of ascertaining the possible effects of this variation in the composition of the *Crotalaria* plant at different growth stages on the formation and accumulation of end-products in the soil, another series of plants was analyzed at different growth stages and decomposition studies were made.

Preparation of material

The plants in this series were grown under field conditions. Sufficient plants were dug at four different growth stages as follows:

1. When the plants were young and succulent.
2. When the first flowers appeared (early reproductive stage).
3. In the advanced seed pod stage.
4. In the late fall or early winter condition (leaves killed by frost).

The plants were taken from the field to the laboratory and separated into the leaves, stems, and roots. Seed pods in the later stages were included with the leaves. These different plant parts were cut into small pieces (one-quarter to one-eighth inch) and dried in a ventilated oven at 70°C. The materials were then ground in a Quaker mill, pulverized in a Dreef mill, and preserved in tightly stoppered bottles for analysis.

Methods of analysis

Sugars. The dried samples were redried in a Cenco vacuum oven at 60°C. The reducing power was then determined on 3-gm. samples, as described in the preceding, after extracting with ether (anhydrous alcohol free) to remove fats, waxes, and resins.

Other carbohydrates. The polysaccharides and other carbohydrates were determined as previously described.

Nitrogen. All forms of nitrogen were determined as described.

Soils studies

The green plant parts were passed through a power food chopper, and 100 gm. of each finely ground plant part were incorporated with 20 pounds of air-dried Norfolk fine sandy soil; 100 gm. of the entire green plant made up of the different plant parts combined in the growth proportions were likewise incorporated. The soils were maintained at an optimum moisture content and incubated under greenhouse conditions.

This procedure was repeated for the plants when they reached the different growth stages mentioned.

Fifty-gram samples of soil were carefully weighed from the duplicate pots in which the soil was composted with the different plant parts and with the whole plant. The nitrate content of the soil was determined by making a 1 to 5 water extract of the soil immediately after sampling and using the phenoldisulfonic acid method. These samples were taken every two weeks after incubation was started. According to Waksman (8), the accumulation of nitrates in the soil may be used as an indication of the decomposition processes or biological activity taking place in the soil. From the nitrate content of the soil in the different pots, the total nitrogen present as nitrate was calculated for each sampling. The amount of nitrogen as nitrate in the soil receiving no additions was subtracted from the amount present in the soil of the green

manured pots and the remainders calculated as percentages of the total nitrogen added in the green manure.

This procedure makes possible a direct comparison between the degree of nitrate accumulation from the different plant parts and from the whole plant when incorporated with the soil at different growth stages, regardless of the fact that the actual quantities of dry matter and nitrogen varied with the plant part and the stage of growth.

A comparison of nitrate accumulation from different plant parts

A comparison of the carbohydrate percentages as given in table 3 with the nitrogen percentages given in table 4, shows striking variations in the ratio between such carbohydrate and nitrogen compounds in the *Crotalaria* plant at different growth stages. This marked variation can be more easily observed by an examination of figures 1, 2, 3, and 4, which represent graphically such carbohydrate and nitrogen percentages in the whole *Crotalaria* plant and its separate parts at different growth stages. The term "carbohydrates" as used on these graphs includes sugars, starches, dextrans, and hemicelluloses or the "easily hydrolyzed carbohydrates" as given in the preceding tables. The differences in the amounts of nitrate accumulation, expressed as the percentage of the total nitrogen added, at the different periods, are also given. A comparison of the differences in the composition of the plant and its parts with the percentages of the total nitrogen added present as nitrates at definite periods after the incorporation of the same with the soil, shows a marked correlation.

The degree of nitrate accumulation from the *Crotalaria* plant and its parts when incorporated with the soil is shown to be in the ascending order; roots, stems, complete plant, and leaves for all the different growth stages. In the early stages of decomposition, the stems and roots show a negative percentage of nitrogen nitrified, which indicates a utilization of the nitrogen (nitrates) of the soil in the decomposition of these plant parts. Conversely, there is a progressive increase in the percentage of nitrogen of the complete plant and of the leaves nitrified for each of these growth stages. These differences in the degree of nitrate accumulation in the soil from the plant and its parts are correlated with differences in the composition of these materials.

As indicated in table 5, the averaged ratios of the total nitrogen to cellulose in the leaves, complete plants, stems, and roots for all the growth stages are 2.0, 7.5, 17.2 and 21.9, respectively. Likewise the ratios of the nitrogen to easily hydrolyzable carbohydrates for the above mentioned materials are 4.5, 9.5, 18.6, 20.5, respectively. Somewhat similar ratios may be noted between other forms of nitrogen and the cellulose and easily hydrolyzable carbohydrates, but the ratios given in table 5 correlate more perfectly with the accumulation of nitrates in the soil after the incorporation of these materials. These ratios between the total nitrogen and the cellulose and easily hydrolyzable carbohydrates in the plant materials correlate with the degree of nitrate accumulation

TABLE 3
Percentage of dry matter, ether extract, easily hydrolyzable carbohydrates, cellulose, and lignin in roots, stems, leaves, and the whole plant of Crotalaria striata at different growth stages during growing season of 1927

(All percentages calculated on dry weight basis except dry matter. All easily hydrolyzed carbohydrates given in terms of glucose)

STAGE OF GROWTH	DRY MATTER				ETHER EXTRACT				REDUCING SUGARS				TOTAL SUGARS			
	Leaves		Stems		Leaves		Stems		Leaves		Stems		Leaves		Stems	
	per cent	Whole plant	per cent	Roots	per cent	Whole plant	per cent	Roots	per cent	Whole plant	per cent	Roots	per cent	Whole plant	per cent	Roots
Succulent.....	16.800	16.000	18.600	16.656	6.655	1.650	1.180	4.026	2.516	2.766	1.333	1.262	7.216	4.066	3.033	5.448
Bloom.....	19.200	22.600	23.200	21.160	6.143	0.846	1.016	3.014	1.683	1.750	0.996	1.615	6.800	2.916	3.033	4.504
Seed pod.....	32.400	38.800	37.800	35.609	4.055	1.273	2.480	2.605	0.966	1.683	1.200	1.325	3.283	2.866	4.016	2.977
Late fall or dormant.....	72.000	70.800	53.500	67.058	1.036	0.866	0.766	0.879	2.000	0.333	0.800	0.730	2.816	2.000	2.273	2.203
	TOTAL POLYSACCHARIDES				TOTAL HYDROLYZED CARBOHYDRATES				CELLULOSE				LIGNIN			
Succulent.....	16.882	16.746	18.732	17.041	24.098	20.812	21.765	22.489	7.424	22.730	24.566	15.643	8.619	18.015	20.198	13.786
Bloom.....	13.748	19.450	18.365	16.987	20.548	22.366	21.398	21.491	8.320	20.686	19.609	15.526	7.891	21.440	22.111	16.049
Seed pod.....	17.949	23.332	21.082	20.761	21.232	26.198	25.098	23.738	12.163	21.066	22.766	17.272	17.233	22.200	22.177	20.141
Late fall or dormant.....	15.182	20.235	19.382	19.130	17.998	22.235	21.655	21.333	8.855	21.650	29.466	20.682	18.200	27.975	22.425	25.122

TABLE 4
*Percentage of dry matter, different forms of nitrogen and total nitrogen in leaves, stems, roots, and whole plant of *Crotalaria striata* at different stages of growth during season of 1927*
 (All nitrogen percentages calculated on a dry weight basis)

STAGE OF GROWTH	DRY MATTER				WATER-EXTRACTED NITROGEN				NON-WATER-EXTRACTABLE NITROGEN				COAGULABLE PROTEIN NITROGEN			
	Leaves		Stems		Roots		Whole plant		Leaves		Stems		Roots		Whole plant	
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Succulent.....	16.800	16.000	18.600	16.656	1.666	0.675	1.010	1.186	3.254	0.885	0.485	1.968	0.205	0.260	0.387	0.248
Bloom.....	19.200	22.600	23.200	21.160	1.416	0.743	0.741	1.020	4.359	0.682	0.409	1.732	0.241	0.085	0.165	0.159
Seed pod.....	32.400	38.800	37.800	35.609	0.802	0.432	0.568	0.607	2.921	0.628	0.325	1.616	0.049	0.021	0.025	0.033
Late fall or dormant.....	72.000	70.800	53.500	67.058	1.022	0.446	0.254	0.518	3.803	0.624	0.771	1.248	0.072	0.018	0.009	0.026
	TOTAL PROTEIN NITROGEN				WATER-SOLUBLE NITROGEN				NITROGEN OF AMINO ACIDS				TOTAL NITROGEN			
Succulent.....	3.459	1.145	0.872	2.216	1.461	0.415	0.623	0.938	0.223	0.241	0.180	0.224	4.920	1.560	1.495	3.154
Bloom.....	4.600	0.767	0.574	2.265	1.175	0.658	0.576	0.861	0.140	0.109	0.087	0.117	5.775	1.425	1.150	2.752
Seed pod.....	2.970	0.649	0.350	1.648	0.753	0.411	0.543	0.564	0.163	0.142	0.221	0.157	3.723	1.060	0.893	2.223
Late fall or dormant.....	3.875	0.642	0.780	1.274	0.950	0.428	0.245	0.492	0.292	0.155	0.079	0.167	4.825	1.070	1.025	1.766

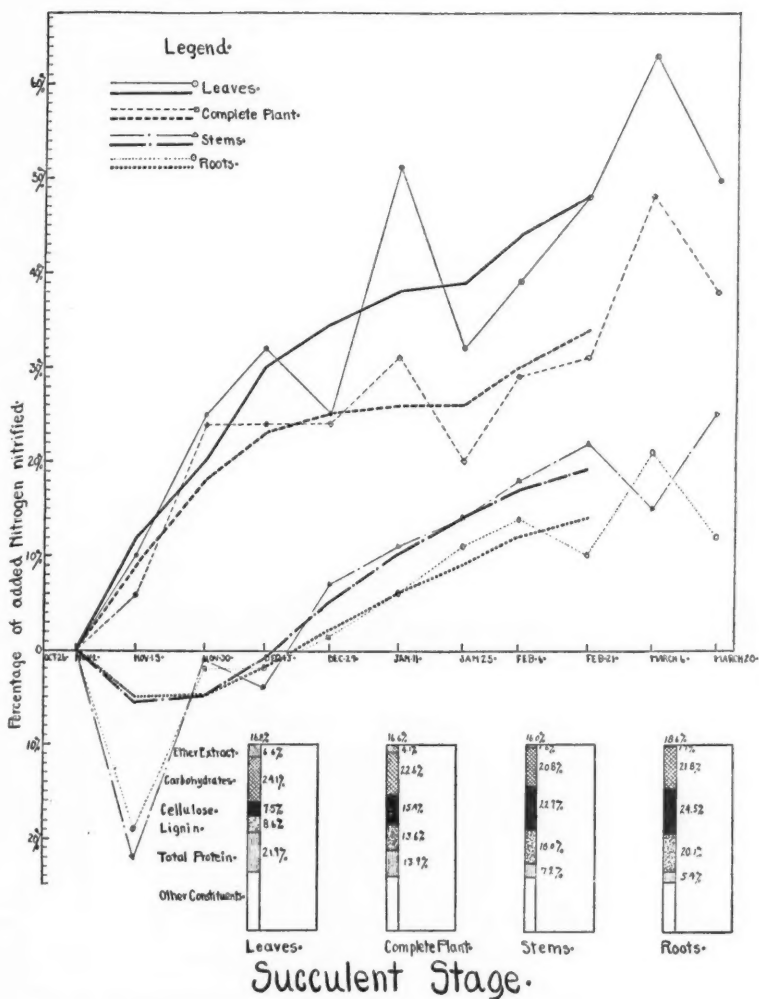


FIG. 1. PERCENTAGES OF NITROGEN NITRIFIED FROM THE *succulent* CROTALARIA PLANT AND ITS SEPARATE PARTS WHEN INCORPORATED WITH NORFOLK SANDY SOIL, AS CORRELATED WITH THE COMPOSITION OF THE PLANT AND ITS PARTS

in the soil: the narrow ratio in the leaves, giving the most rapid accumulation of nitrate, contrasted with the slower accumulation of nitrates for the soil, containing stems or roots with a wide ratio, in which an actual nitrogen deficit occurred.

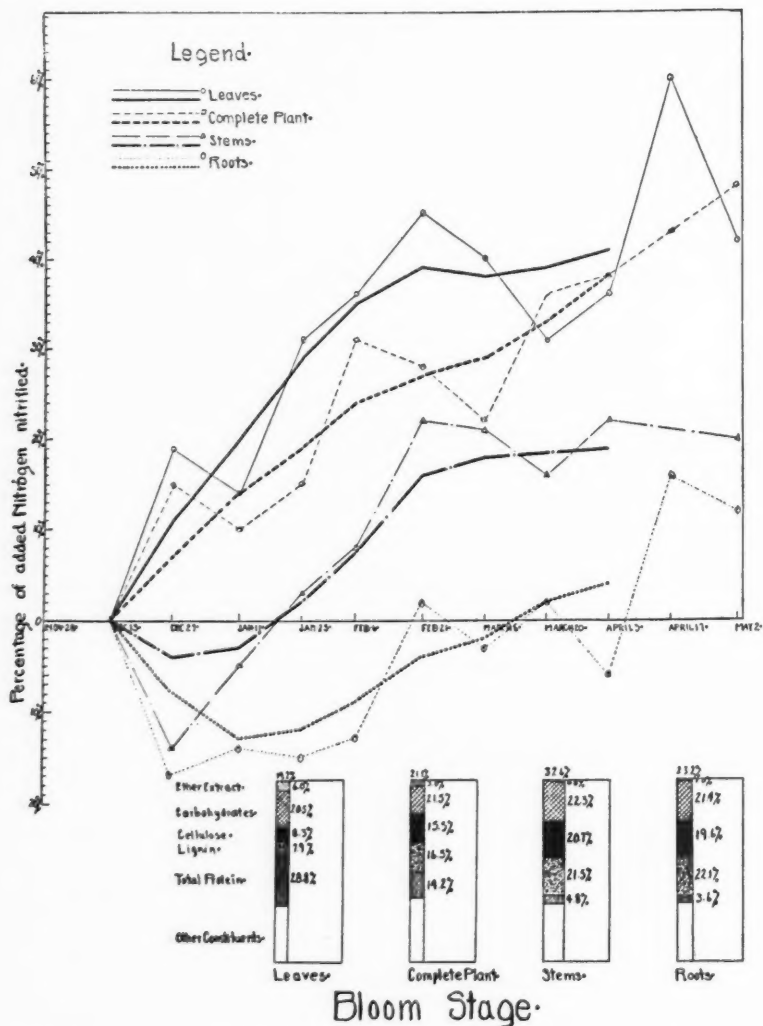


FIG. 2. PERCENTAGES OF NITROGEN NITRIFIED FROM THE COMPLETE CROTALARIA PLANT AND EACH OF ITS PARTS WHEN INCORPORATED WITH NORFOLK SANDY SOIL AS CORRELATED WITH THE COMPOSITION OF THE PLANT AND EACH OF ITS PARTS IN THE EARLY bloom stage OF GROWTH

These results are in keeping with the findings of Waksman and Tenney (9) who have made a study of the decomposition of the rye plant at different stages of growth, using CO_2 evolution as a measure of decomposition. These authors

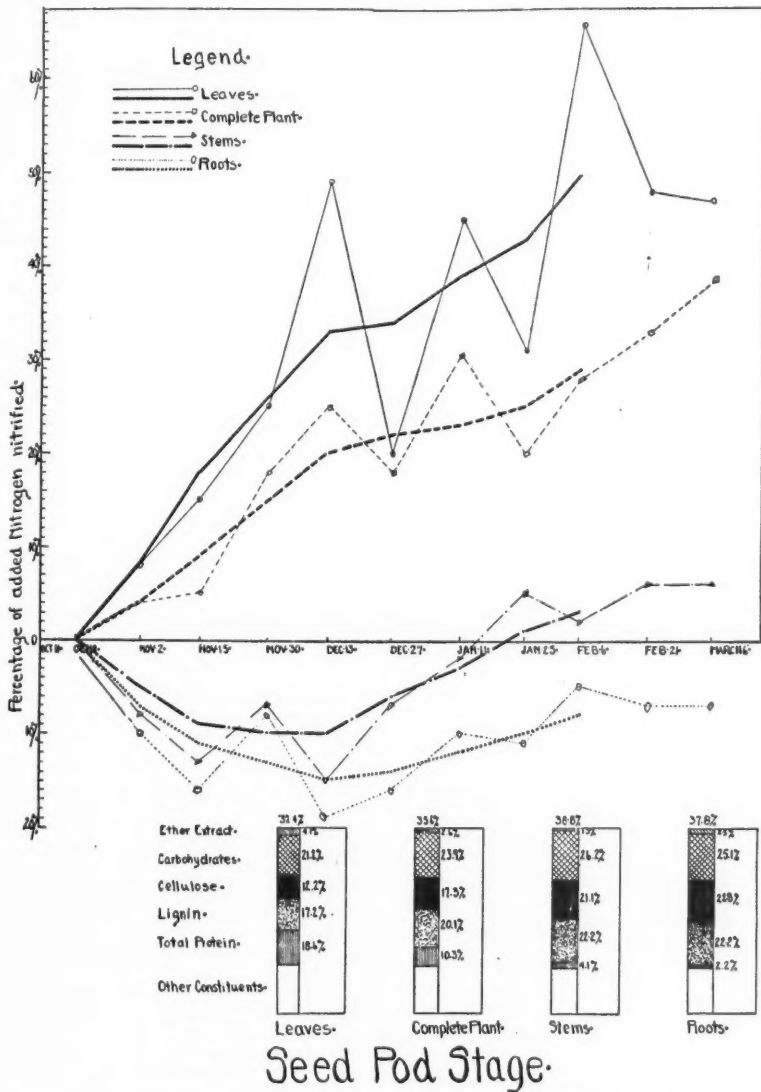


FIG. 3. PERCENTAGES OF NITROGEN NITRIFIED FROM THE COMPLETE CROTALARIA PLANT AND EACH OF ITS PARTS WHEN INCORPORATED WITH NORFOLK SANDY SOIL AS CORRELATED WITH THE COMPOSITION OF THE PLANT AND EACH OF ITS PARTS IN THE *seed pod stage* OF GROWTH

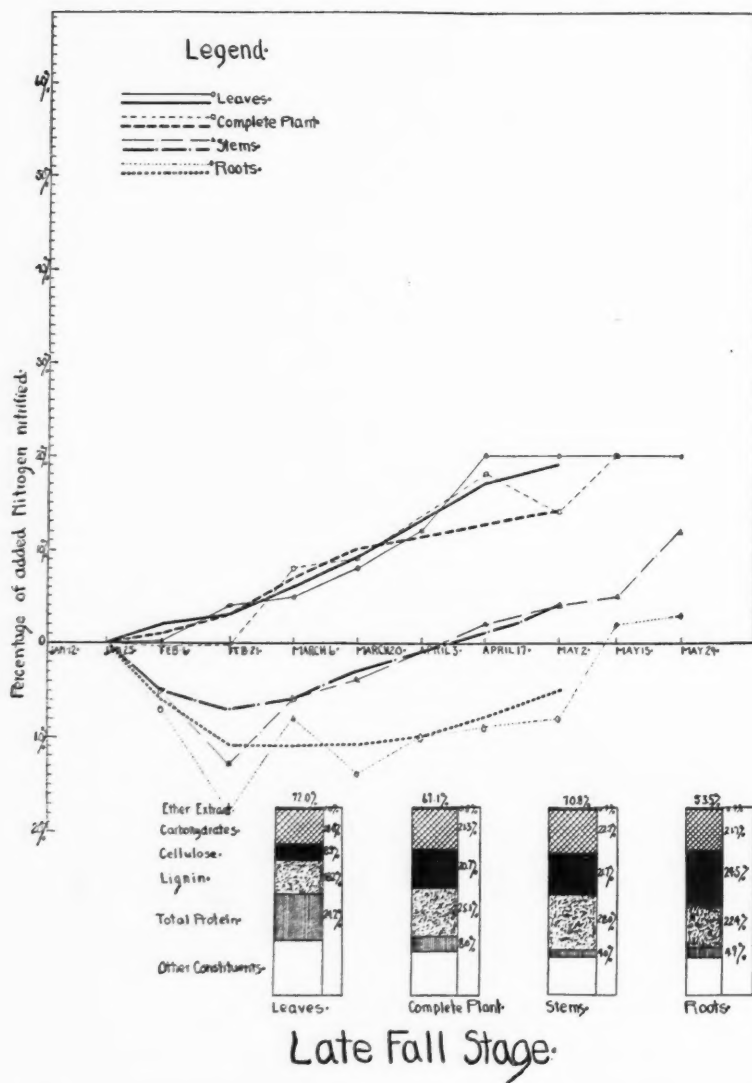


FIG. 4. PERCENTAGES OF NITROGEN NITRIFIED FROM THE COMPLETE CROTALARIA PLANT AND EACH OF ITS PARTS WHEN INCORPORATED WITH NORFOLK SANDY SOIL AS CORRELATED WITH THE COMPOSITION OF THE PLANT AND EACH OF ITS PARTS IN A late fall growth stage OR AFTER FIRST FROST

maintain that 1.7 per cent nitrogen in the dry matter is just sufficient to cover the requirement of the microorganisms which are active in the decomposition of the plant (rye) material within a period of four weeks. Further, they contend that if the plant contains more than 1.7 per cent nitrogen, the excess nitrogen is rapidly liberated in an available form even within the first four weeks of decomposition. Thus, the complete *Crotalaria* plant and the *Crotalaria* leaves with a higher percentage of nitrogen than that mentioned in the foregoing, show a decidedly rapid accumulation of nitrate during the entire decomposition period, while the stems and roots with a percentage of nitrogen lower than 1.7 per cent, show an actual utilization of soil nitrogen during the early stages of decomposition.

This rate of nitrate accumulation in soil manured with the different plant parts is further substantiated by results by Joshi (4). This worker obtained

TABLE 5
Ratios of nitrogen to cellulose and easily hydrolyzable carbohydrates

STAGE OF GROWTH	RATIO TOTAL NITROGEN TO CELLULOSE				RATIO TOTAL NITROGEN TO EASILY HYDROLYZABLE CARBOHYDRATES			
	Leaves	Complete plant	Stems	Roots	Leaves	Complete plant	Stems	Roots
Succulent.....	1:1.5	1: 4.7	1:14.5	1:16.4	1:4.9	1: 7.1	1:13.0	1:14.3
Early bloom.....	1:1.4	1: 5.6	1:14.5	1:17.0	1:3.6	1: 7.9	1:15.7	1:18.6
Seed pod.....	1:3.2	1: 7.8	1:19.8	1:25.4	1:5.7	1:10.7	1:24.7	1:28.1
Late fall.....	1:1.8	1:11.7	1:20.2	1:28.7	1:3.7	1:12.1	1:20.8	1:21.1
Average.....	1:2.0	1: 7.5	1:17.2	1:21.9	1:4.5	1: 9.5	1:18.6	1:20.5

a more rapid accumulation of nitrates in the soil from the incorporation of leaves of sann-hemp (*Crotalaria juncea*) than from the roots and stems of the same plant at one stage of growth. He also obtained similar results with dhaincha, guvar, and cowpeas. Joshi (4) indicated that the higher nitrogen content of the leaves of these plants was responsible for the more rapid accumulation of nitrates following their incorporation with the soil.

Bal (2), studying the decomposition of sann-hemp (*Crotalaria juncea*) at different stages of growth, found that the leaves decomposed more rapidly than did the stems of this plant. Studies of the nitrate accumulation from the complete plant as compared with that from the leaves and stems added to the soil separately showed that the leaves decomposed more rapidly than the complete plant, which decomposed more rapidly than the stems. In analyzing the data in the decomposition of the complete plant, Bal, calculating the relative percentage of nitrogen in the complete plant which was obtained from the stems and leaves and utilizing the results obtained on the incorporation of these plant parts separately, arrived at the conclusion that the stems had no influence upon the nitrate accumulation from the leaves when the stems and leaves were added together (as a complete plant) to the soil.

By a similar calculation, the roots, stems, and leaves of *Crotalaria striata* when incorporated with the soil separately and when added to the soil together (as a complete plant) produced nitrate accumulations analogous to those reported by Bal. Table 6 gives the percentages of the total nitrogen nitrified from the complete plant as observed experimentally together with the calculated percentages, the figures obtained upon the incorporation of the individual plant parts being utilized separately. Within the limits of experimental error, these calculated figures indicate that the roots and stems do not decrease the degree of nitrate accumulation from the leaves at the various growth stages of *Crotalaria striata*. Apparently the nitrogen (nitrates) of the original soil, which, as stated in the foregoing, is taken into consideration in these calculations, is sufficient to supply the needs of the organisms for the decomposition of the roots and stems.

The change in the degree of nitrate accumulation through the successive growth stages

The degree of nitrate accumulation in soils incorporated with the complete *Crotalaria* plant or any one of its parts appears to be associated with the ratio of nitrogen to carbohydrate materials, i.e., easily hydrolyzable carbohydrates and cellulose. Consequently, the change in the rate of nitrate accumulation in the soil incorporated with the complete plant or any one of its parts should vary at successive growth stages with the deviation in the composition of the plant or its parts through these growth stages.

A high rate of nitrification took place in the soil when it was incorporated with *Crotalaria* leaves in the succulent growth stage (fig. 5). A very similar rate of nitrification is obtained when the soil was manured with leaves of the *Crotalaria* plant in the early bloom stage or with those from the plant in the late seed pod stage. This increased degree of nitrate accumulation appears to be associated with a narrow ratio between the nitrogen and carbohydrate materials (easily hydrolyzable carbohydrates, cellulose) in the leaves. A similar ratio between the nitrogen and carbohydrates was found in the leaves of the plant in its late fall condition—after frost—but the degree of nitrification in the soil manured with these leaves was somewhat less than that of those from the plants in the first three growth stages. This difference is undoubtedly associated with the decreased percentage of plant moisture in the latter leaves.

This dependence of the degree of nitrate accumulation in the soil from plant material incorporated therein on the moisture content of such plant material is further substantiated by Hutchinson and Milligan (3) from whom we quote as follows:

The successful decomposition of the buried green crop has been shown to be determined largely by the presence of an adequate supply of water for the needs of the saprophytic organisms upon which this process depends. It is therefore to be expected that the moisture content of the green plant at the time of burial will materially affect the result, as will also the condition of the tissues in this respect. Thus, the decomposition of the younger plants is

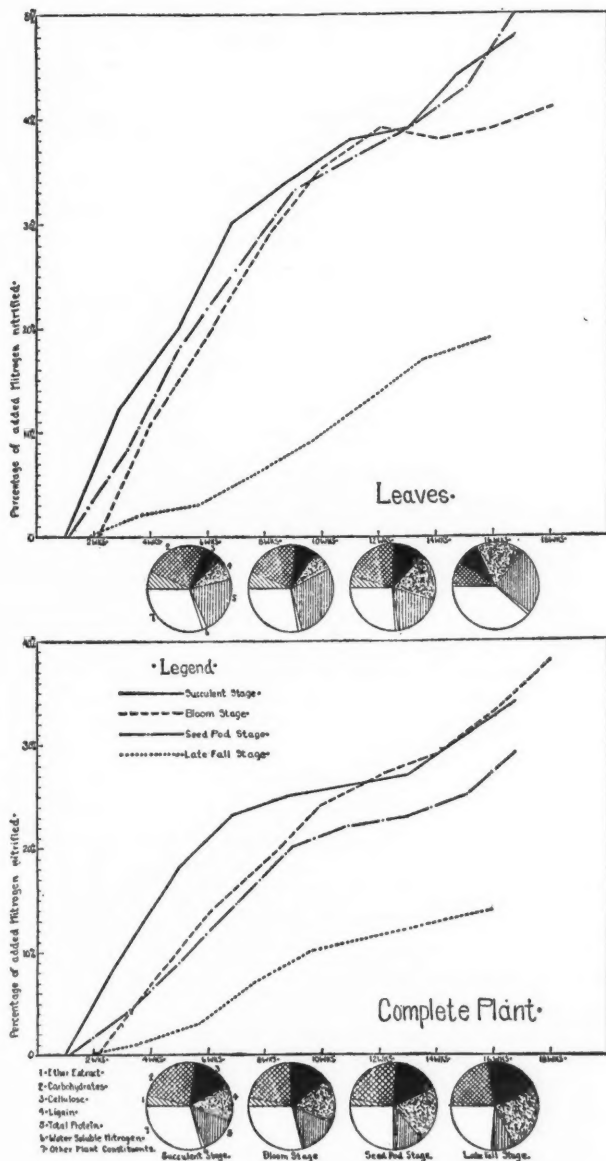


FIG. 5. PERCENTAGES OF NITROGEN NITRIFIED FROM THE LEAVES AND THE COMPLETE PLANT WHEN INCORPORATED WITH NORFOLK SANDY SOIL AT DIFFERENT GROWTH STAGES AS CORRELATED WITH THE COMPOSITION OF THE PLANT AT SUCH GROWTH STAGES

as much more rapid than that of the maturer ones as the water content of the former is higher than that of the latter, and those parts of the plant containing the highest proportion of cell sap have been found to decompose more rapidly than the maturer portions. This is well illustrated in the relative rates of nitrification of the plants in the three stages of growth under observation, and has a further bearing upon the effect of loss of moisture from the plants resulting from delay in burying after cutting down.

Whiting and Schoonover (10) observed that the decrease in the degree of nitrification from dehydration appears to be of a physical nature only.

The degree of nitrate accumulation in the soil manured with the complete plant at the successive stages of growth shows a progressive decrease with advancing maturity of the plant (fig. 6). This progressive decrease in the rate of nitrate accumulation is associated with a widening of the ratio between the nitrogen and the carbohydrate material (easily hydrolyzable carbohydrates, cellulose) from that in the succulent stage to that in the late fall stage, or for nitrogen to cellulose from 4.7 (succulent stage) to 5.6 (early bloom) to 7.8 (seed pod), to 11.7 (late fall), or for nitrogen to easily hydrolyzable carbohydrates from 7.1 (succulent stage) to 7.8 (early bloom stage) to 10.7 (seed pod) to 12.1 (late fall). These changes in composition are chiefly found in the stems and roots of the plant.

The time which elapses before nitrate accumulation takes place in the soil upon the incorporation of stems and roots of the plant of different growth stages is correlated with the width of the ratio between the total nitrogen and carbohydrates (see fig. 6 and Table 5). After the elapse of this period necessary for a positive accumulation of nitrates, there is a progressive increase in the degree of nitrate accumulation for the stems and roots of each of the different growth stages of the plant, the more rapid accumulation taking place with those stems and roots having a narrower ratio of nitrogen to carbohydrate material (succulent and early bloom stage).

Immediately after the incorporation of the stems and roots of the *Crotalaria* plant, a rapid and marked utilization of the nitrates of the soil is apparent. Subsequently, the amounts of nitrates utilized by the organisms in their decomposition of the plant materials gradually decrease. It appears that this early and large utilization of the nitrates of the soil is associated with the decomposition of the easily hydrolyzable carbohydrates of the stems and roots; subsequently to this the cellulose decomposing organisms utilize the nitrates until the ratio of nitrogen in the decomposing plant material to the microbial bodies is such that a positive accumulation of nitrates occurs.

Recovery of nitrogen

The accumulation of nitrates in the soil resulting from the incorporation of the *Crotalaria* plant or any one of its parts at the different growth stages is further illustrated by the response of the growths of Sudan grass in the soils thus treated. All the soil pots in which nitrate accumulation studies were made after the incorporation of the complete plant or any one of its parts,

were seeded with Sudan grass. Five plants were grown in each pot. Successive cuttings of this grass were made during the season at the maximum growth stage. The total yields from the pots are recorded in table 7. The percentage of nitrogen on a sample taken from the accumulated yields from each pot was determined. The percentage of nitrogen recovered in the Sudan grass from the different pots in which the soil had been manured with the complete plant or any one of its parts is likewise tabulated. From these data, it may be observed that the recovery of nitrogen in the Sudan grass is correlated with the degree of nitrate accumulation in the soil treated with the complete plant or any of its parts. This in turn is again correlated with the composition of the complete plant or any one of its parts at the different growth stages.

TABLE 6

Calculated and observed percentages of nitrogen nitrified when complete plant is incorporated with the soil

TIME AFTER INCORPORATION	SUCCULENT STAGE		BLOOM STAGE		SEED POD STAGE		LATE FALL STAGE	
	Calculated*	Observed	Calculated*	Observed	Calculated*	Observed	Calculated*	Observed
weeks	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
2	7.7	9.4	6.9	7.3	4.5	4.0	-1.4	0.9
4	13.9	17.5	13.7	13.6	10.8	9.3	-2.2	2.7
6	22.2	22.9	21.5	19.1	16.4	15.1	-0.3	6.7
8	26.7	25.1	27.7	23.7	21.6	19.9	2.4	10.4
10	30.5	25.9	32.2	26.9	23.2	21.8
12	32.4	25.9	32.2	29.3	27.9	23.4
14	35.9	29.6	31.8	24.5	8.4
16	40.7	33.8	34.6	37.7	37.3	28.6	10.9	14.43

* Calculated from the percentages of nitrogen nitrified when the leaves, stems, and roots were incorporated separately and from the percentages of nitrogen in the complete plant in the form of these plant parts.

APPLICATION

The utilization of the *Crotalaria* plant for the sandy soils with a low organic matter content is becoming a general farm and grove practice. The introduction of this summer leguminous plant has created a number of problems relative to its proper use in the numerous farm and grove practices. Since the accumulation of nitrogen in the soil subsequent to the incorporation of a green manure crop must coincide with the maximum demand for nitrogen of the crops to be benefited, it follows that the planting and turning under of a green manure crop must be adjusted to the various cultural practices of such crops.

All other conditions being favorable, if a rapid accumulation of nitrates is desired, as in the case of vegetable crops, then the *Crotalaria* plant should be incorporated with the soil in its early growth stages. If, on the other hand, a retarded accumulation of nitrate is desired, so that the crop or tree may make use of the nitrogen in the spring months, the plant should be in an advanced

stage of growth and dry before it is incorporated with the soil. The present study of the composition relation of the *Crotalaria* plant to the accumulation of nitrates in the soil may be of value in the adjustment of this leguminous plant in the many varied cultural practices in which it will no doubt be used.

TABLE 7

The yield of dry matter, percentages of nitrogen in the dry matter, and percentages of added nitrogen recovered in Sudan grass planted on soils receiving green manures in the form of Crotalaria leaves, complete plants, stems, and roots at different growth stages

PLANT OR PLANT PARTS INCORPORATED	AVERAGE YIELD OF SUDAN GRASS	AVERAGE NITROGEN IN GRASS	NITROGEN RECOV- ERED	INCREASED NITROGEN RECOV- ERED	NITROGEN ADDED AS GREEN MANURE	NITROGEN RECOV- ERED
	gm.	per cent	gm.	gm.	gm.	per cent
<i>Succulent stage</i>						
Nothing.....	6.5	0.605	0.039
Leaves.....	34.9	0.990	0.346	0.307	0.827	37.1
Complete plant.....	34.1	0.833	0.284	0.245	0.531	46.1
Stems.....	19.9	0.535	0.106	0.067	0.250	26.8
Roots.....	19.9	0.508	0.101	0.062	0.279	22.2
<i>Bloom stage</i>						
Nothing.....	6.5	0.598	0.039
Leaves.....	35.3	1.258	0.444	0.405	1.108	36.6
Complete plant.....	33.8	0.795	0.269	0.230	0.665	34.6
Stems.....	23.2	0.620	0.144	0.105	0.321	32.7
Roots.....	16.8	0.609	0.102	0.063	0.267	23.6
<i>Seed pod stage</i>						
Nothing.....	6.5	0.605	0.039
Leaves.....	44.9	1.038	0.466	0.427	1.205	35.4
Complete plant.....	32.3	0.873	0.282	0.243	0.793	30.6
Stems.....	17.9	0.583	0.104	0.065	0.411	15.8
Roots.....	14.1	0.533	0.075	0.036	0.336	10.7
<i>Late fall stage</i>						
Nothing.....	14.7	0.604	0.089
Leaves.....	56.8	1.150	0.653	0.564	3.470	16.3
Complete plant.....	34.9	0.960	0.335	0.246	1.194	20.6
Stems.....	27.9	0.823	0.230	0.141	0.758	18.6
Roots.....	14.3	0.559	0.080	0.546	0.0

All results averages of duplicate treatments.

SUMMARY

A study has been made of the composition of the *Crotalaria* leaves, stems, roots, and complete plant at different stages of growth with special reference to nitrogen and carbohydrate compounds. The plants were analyzed in the

succulent, bloom, seed pod, and late fall growth stages. A study of the course of the accumulation of nitrates in Norfolk sandy soil upon the incorporation of the complete plant and each of its parts was made.

In any growth stage, there is a progressive decrease in percentage of nitrogen in the complete plant and its separate parts in the following order: leaves, complete plant, stems, and roots. There is a progressive increase in the percentage of cellulose and lignin in the order: leaves, complete plant, stems, roots. The carbohydrate percentage in the plant and its parts was rather constant. This resulted in a narrow ratio between total nitrogen and carbohydrates and cellulose for the leaves, which gradually widened in the complete plant, stems, and roots, respectively.

The accumulation of nitrates following the incorporation of the complete plant or any of its parts was most rapid from the leaves, progressively decreasing from the complete plant, stems, and roots in the order named, except with the leaves in the late fall stage, which varied little from that of the complete plant. The stems and roots show a utilization of the nitrate nitrogen of the soil in the early stages of decomposition. There is relatively little variation in the percentages of nitrogen and carbohydrates in the leaves of the plant through the successive growth stages but there is some increase in the percentages of lignin and cellulose as the plant approaches maturity. There is little variation in the rapidity of the nitrification of the nitrogen added as leaves, with the exception of the leaves in the late fall growth stage, where desiccation occurred. With the complete plant through the successive growth stages, there is a progressive increase in percentage of lignin and cellulose with a corresponding decrease in percentage of nitrogen, whereas the percentage of the carbohydrates remains rather constant.

There is a progressive decrease in the rapidity of the nitrification of the nitrogen added to the soil from the complete plant through the successive stages of growth, i.e., succulent, bloom, seed pod, and late fall, in decreasing order.

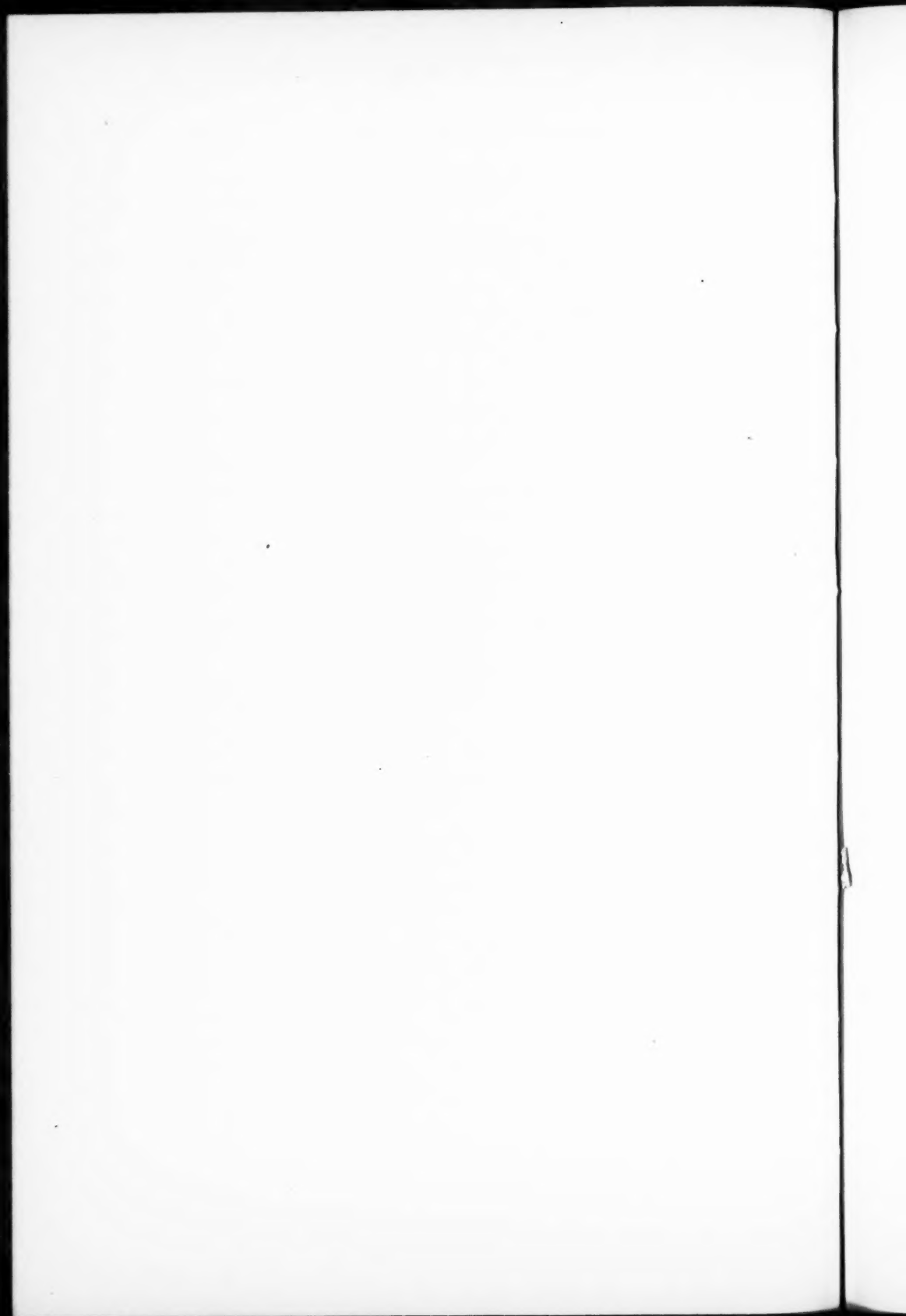
In the first two growth stages (succulent and bloom) there is little variation in the percentage of nitrogen, cellulose, and lignin in the stems and roots, whereas during the two latter growth stages there is an increase in percentage of cellulose and lignin and a decrease in the percentage of nitrogen. The carbohydrates were rather constant in percentage for all growth stages.

There is only a slight variation in the course of nitrification of nitrogen added to the soil as stems in the succulent and bloom stages. This course showed a more rapid accumulation of nitrates from these stems than from the stems of the seed pod and late fall stages. The time required for a positive accumulation of nitrates after the incorporation of the stems was shorter for the succulent and bloom stage stems than for the seed pod and late fall stems. Similar results were obtained with the roots.

Possible practical applications for the foregoing results are given.

REFERENCES

- (1) Association Official Agricultural Chemists. 1924 Devardo Method Official and Tentative Methods of Analyses, p. 12. Washington, D. C.
- (2) BAL, D. V. 1922 Studies on the decomposition of some common green-manuring plants at different stages of growth in the black cotton soil of the central provinces. *Agr. Jour. India*. 17: 133-155.
- (3) HUTCHINSON, C. M., AND MILLIGAN, S. 1914 Green manuring experiments, 1912-13. Agr. Res. Inst., Pusa, Bul. 40.
- (4) JOSHI, N. V. 1919 Rate of nitrification of different green manures and parts of green manures and the influence of crop residues on nitrification. *Agr. Jour. India*. 14: 395-413.
- (5) KRAUS, E. J., AND KRAYBILL, H. R. 1918 Vegetation and reproduction with special reference to the tomato. Ore. Agr. Exp. Sta. Bul. 149.
- (6) SCHAFER, P. A., AND HARTMAN, A. F. 1921 The idiometric determination of copper and its use in sugar analysis. *Jour. Biol. Chem.* 45: 349-390.
- (7) STOKES, W. E. 1927 Crotalaria as a soil-building crop. *Jour. Amer. Soc. Agron.* 19: 944-948.
- (8) WAKSMAN, S. A. 1923 Microbiological analysis of soils as an index of soil fertility: V. Methods for the study of nitrification. *Soil Sci.* 15: 241-260.
- (9) WAKSMAN, S. A., AND TENNEY, F. G. 1927 The composition of natural organic materials and their decomposition in the soil: II. Influence of age of plant upon rapidity and nature of its decomposition—rye plants. *Soil Sci.* 24: 317-333.
- (10) WHITING, A. L., AND SCHOONOVER, W. R. 1920 The comparative rate of decomposition of green and cured clover tops in soil. *Soil Sci.* 9: 137-149.
- (11) WILEY, H. W. 1914 Principles and Practice of Agricultural Analysis, ed. 2, vol. 3, p. 12. New York.



THE LAWS OF SOIL COLLOIDAL BEHAVIOR: II. CATAPHORESIS, FLOCCULATION, AND DISPERSION¹

SANTE MATTSON

New Jersey Agricultural Experiment Station

Received for publication May 1, 1929

In the study of colloidal behavior the problem of stability is of fundamental importance. It is generally assumed that the stability of a suspension is directly connected with the potential difference within the double layer. This represents the cataphoretic potential ζ which may be calculated from the cataphoresis of the particles according to the Helmholtz-Perrin formula:

$$\zeta = \frac{4 \pi \eta v}{H D}$$

where v = velocity of the particles, η = viscosity of liquid, H = potential gradient in volt/cm., and D = dielectric constant of liquid.

With $\eta = 0.01$ and $D = 81$ for water at room temperature, the potential expressed in millivolts will be found by multiplying the velocity of the particles, expressed in μ per second, by the factor 14. It has recently been brought out however, by Debye and Hückel (6) that for spherical particles the factor 4 in the above equation should be 6. This would make the cataphoretic potentials reported in earlier papers 50 per cent larger. Multiplying the velocity by 21 instead of by 14 would therefore include this correction. It is in all cases best to report the observed quantities, i.e., the velocities.

In connection with the foregoing work it was deemed of utmost importance to determine the influence of the different electrolytes on the stability of the suspensions and on the cataphoretic migration. This was done as reported in previous publications (21) a cataphoresis cell of the same construction being employed.

THE EFFECT OF BASES AND SALTS

Table 17 shows the cataphoresis of bentonite in various concentrations of the chloride, sulfate, and ferrocyanide of sodium. The concentrations at which the suspensions flocculated are indicated by x 's, one x signifying a beginning of flocculation while four x 's stand for complete flocculation. The suspensions were prepared by mixing 25 cc. of a 0.2 per cent stock suspension with 25 cc. of the solutions. After standing over night the flocculation was observed, the

¹ Paper of the Journal Series, New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

tubes were shaken, and the cataphoresis measurements made. The latter represent an average of three measurements in each direction, the current being reversed each time.

Let us first consider the flocculation. In a concentration of N 0.02, flocculation was complete in the chloride, about half complete in the sulfate, and had not begun in the ferrocyanide solution. In N 0.03 it was complete in the sulfate and about half complete in the ferrocyanide solution. In the ferrocyanide solution flocculation was complete in the N 0.04 concentration. There is therefore an unmistakable valence effect and the most significant fact is that this effect agrees, one could almost say quantitatively, with the suppression of the Donnan potential, the swelling, and the viscosity, as shown in part I of this paper. Flocculation being in effect a suppression of the stability, one is led to

TABLE 17
Flocculation and cataphoresis of bentonite

CONCENTRATION	NaCl			Na ₂ SO ₄			Na ₂ Fe(CN) ₆		
	Flocculation	μ /sec. 1 volt/cm.	Millivolts calculated	Flocculation	μ /sec. 1 volt/cm.	Millivolts calculated	Flocculation	μ /sec. 1 volt/cm.	Millivolts calculated
N									
0.0	0	-2.77	-58.2
0.001	0	-2.80	-58.8	0	-3.10	-65.0	0	-3.36	-70.5
0.004	0	-2.85	-59.8	0	-3.16	-66.4	0	-3.52	-74.0
0.01	0	-2.85	-59.8	0	-3.33	-70.0	0	-3.52	-74.0
0.02	xxxx	-3.16	-66.4	xx	-3.61	-75.7	0	-3.79	-79.5
0.03	xxxx	-3.53	-74.0	xxxx	-3.87	-81.3	xx	-3.93	-82.5
0.04	xxxx	-3.73	-78.3	xxxx	-3.96	-83.1	xxxx	-4.03	-84.5

the conclusion that a suppression of one is accompanied by a suppression of the other of these phenomena and that they must all be closely related to one another.

A quantitative relationship of the valence of the ions with the same sign of charge as the colloid has never been established, for, although it is generally admitted that the nature of the ions with the same sign of charge does influence the stability, attention has chiefly been focused on the ions of opposite sign, the valence effect of which are usually much more pronounced. As far as the Donnan equilibrium has anything to do with the action of electrolytes on the stability of colloids, nothing but the valence of the ions is of importance. In the case of an electronegative colloid the equivalent concentration of an electrolyte with a divalent anion would have to be about 50 per cent greater than the concentration of an electrolyte with a monovalent anion (the cation being the same) in order to produce the same effect. This is approximately the relationship between the flocculating concentrations of the chloride and the sulfate solutions in the preceding experiment. In the case of a solution with a divalent cation only approximately one-half the equivalent concentration of a

solution with a monovalent cation (the anion being the same) should be necessary to produce the same effect as far as the influence of the Donnan equilibrium is concerned. The exact relationship will depend on the ratio $\frac{z}{y}$.

The first relationship, that of the anions, can only hold when the anions do not enter into combination with the colloid, as in the preceding case. It is only the free, uncombined fraction of the electrolyte which takes part in the Donnan distribution. The second relationship never holds. The flocculating power of a divalent cation is usually ten to twenty times as great as that of a monovalent cation. (We continue our reference to a negative colloid.) The reason is that the cation, the ion of opposite sign of charge, always combines with the colloid by exchange. Each new combination yields a new complex, a different micelle and a different colloid having different properties. In these combinations any property of the ions may be of importance, such as size, electro-affinity, activity, hydration, and valence. It is in this relationship that the Hofmeister or lyotropic ion series become manifest. We can no more deny the influence of these series than we can deny the influence of the Donnan equilibrium—both are present but very often the effect of one will be found to obscure the effect of the other.

Comparing the cataphoretic potential in the three solutions at the concentrations sufficient to flocculate we do not find a constant critical potential as is generally assumed on the basis of the much quoted experiment of Powis (26) on an oil emulsion. The critical cataphoric potential is lowest in the chloride solution and highest in the ferrocyanide. As far back as 1920 while working in the laboratories of Professor P. Ehrenberg in Göttingen the author (15) established this anomaly for the first time. These observations have recently been verified by other investigators (12, 28). Numerous experiments, some of which will be here presented, show that, as far as soil colloids are concerned, there is no one critical cataphoretic potential but that this potential is different for each pair of ions.

Following the work of Hardy (10) it was believed and has, until recently, frequently been stated that flocculation takes place only at the isoelectric point. But the work of Burton (3), Powis, and others later showed that the flocculating particles retained a considerable part of their charge. The view that the stability is directly related to the cataphoretic potential is accepted by most authorities although it is not clear how the P. D. between two concentric layers can give rise to an external repulsion. Porter and Hedges explain how the mutual action between the particles may be of the nature of an attraction instead of a repulsion. They state (25):

When the existence of this double layer is recognized, the electrical forces between the particles become zero, except in so far as relative displacement takes place by induction between two members of a layer so as to give it an electrical moment. In this case the force between two such doublets in the equilibrium state will, on the average, be an attraction and not a repulsion.

In order to connect the charge of the double layer directly with the stability, it is argued that the spheres of like signs are unable to penetrate one another and thus prevent actual collision of the particles. But is flocculation the result of such actual collisions? Consider the large volume occupied by the flocculated material, which may amount to over 100 cc. per gram solids (1). Or consider the setting into a gel of a 5.36 per cent suspension of bentonite when sufficient NaOH was added to cause flocculation (*compare* table 16, part I). Dividing by 2.65, the approximate sp. gr., we find that the solid particles occupy only a little over 2 per cent of the gel. The particles must, therefore, be comparatively far apart from one another, yet they are linked together forming a continuous, more or less rigid network. This shows definitely that it must be the bulky micelles and not their nuclei, i.e., the particles themselves, which adhere in the process of flocculation. In other words, linkage is established between the outer spheres of the micelles, that is, in the very regions in which the repelling force is supposed to reside.

Another irregularity from the point of view of the generally accepted theory on stability appears in table 17, in that the cataphoretic P.D., as calculated from the speed of migration, is higher in the case of the flocculated material than in the absence of electrolytes. This phenomenon was first observed by Anderson and Mattson when studying the effect of methylene blue on the same sample of bentonite (1). We shall meet with similar cases in connection with soil colloids later.

An increase in the charge in low concentrations of the salts of the alkali metals has quite generally been met with. The logical interpretation of this behavior has been the assumption that the anion is more extensively adsorbed in low concentrations, whereas in higher concentrations the cation adsorption gradually catches up with the adsorption of the anion. This would nicely explain the rise and fall in the migration velocities but it is not in harmony with the observations here made that neither the Cl , NO_3 , SO_4 or $\text{Fe}(\text{CN})_6$ ions are adsorbed by the colloid. On the contrary, we have seen that these ions are negatively adsorbed because of the Donnan distribution. They therefore can not cause an increase in the cataphoretic potential by a preferential adsorption. Neither is the assumption that the cation adsorption gains on the anion adsorption in higher concentrations, in harmony with the foregoing results, which show that the micellar cation concentration, i.e. the value of z , increases with the concentration of the added electrolyte. This release of cations must give rise to a greater number of free charges on the surface of the particle and should, according to formula (C), increase the cataphoretic potential, provided the thickness of the double layer is not at the same time decreased.

The question arises: Is the suppression of the stability caused by the free uncombined ions or it is caused by an adsorption of ions of opposite charge? If the suppression is related to the Donnan equilibrium, as is the suppression of the swelling viscosity and the Donnan potential, then it is the free electrolyte which is the deciding factor. In as far as the suppression of the stability

depends on a neutralization of the free charges on the surface of the particles, we must look for an explanation in the adsorption, i.e. in the association tendency of the ions of opposite sign of charge. We know that the P.D. between the micellar solution and the outside liquid, i.e. the Donnan potential, must be proportional to the micellar ion concentration, i.e., to the number of cations dissociated by the particles. The divalent cations are less dissociated than the monovalent cations and accordingly give rise to a lower P.D. This is in harmony with the dispersibility of soil materials saturated with different cations. In the case of Na-, K-, Mg-, Ca-, and Ba-saturated soil samples, Gedroiz (8) found the following order in the amount of material dispersed $\text{Na} > \text{K} > \text{Mg} > \text{Ca} > \text{Ba}$. As to the *suppression* of the Donnan P.D. by the addition of electrolytes, we know that, apart from any change in the nature of the micelle by an exchange of cations, the free electrolyte suppresses the P.D. according to the valence of the ions and in proportion to the concentration, as has been shown.

By taking into account the different tendencies of the various cations to remain dissociated in the micellar solution and by recognizing the valence effect on the Donnan distribution of the free electrolyte, we can easily formulate a theory which will as satisfactorily explain the stability of a suspension as we are able to explain the swelling and the viscosity. In the above experiment the cation (Na) of the added electrolytes was the same as that in the colloid. The cation influence was therefore the same in all cases. The influence of the valence of the anions, none of which were adsorbed, was almost quantitatively what it should be if the Donnan equilibrium were the sole factor. But so far nothing explains the anomalies of the cataphoretic potential. Before proceeding with the discussion it will therefore be profitable to give some additional data on flocculation and cataphoresis.

Table 18 shows the effects of KCl on the untreated, the electro dialyzed, the Na-saturated, and the Ca-saturated Sharkey clay soil colloid.

The electro dialyzed colloid flocculated in a concentration of KCl about four times weaker than the concentrations in which the base-saturated sample flocculated. This greater sensitiveness of the unsaturated (H-saturated) sample is probably due to the exchange acidity, which mobilizes the trivalent cations Al and Fe (22). This emphasizes the importance of the condition of the colloid. Comparable results can only be obtained by working with material saturated with the same cation as that of the electrolyte employed. There is here no apparent difference between the stability of the Na- and Ca-saturated suspensions, but that is chiefly because the suspensions were so dilute that the small quantity of Ca ions displaced by the K ions did not materially affect the flocculation. In more concentrated suspensions the effect of cation exchange will be pronounced.

The influence of a displacement of Ca ions by K ions shows itself however in the P.D., which increases from 40.1 to a maximum of 50.6 millivolts in the case of the Ca-saturated colloid. In all other cases the P.D. decreases with an

TABLE 18
Sharkey soil colloid—KCl System
 (0.4 gm. colloid per liter)

MILLIEQUIVALENTS IN LITER	FLOCCULATION	$\mu/\text{SEC.}$ 1 VOLT/CM.	P.D. MILLI-VOLTS	FLOCCULATION	$\mu/\text{SEC.}$ 1 VOLT/CM.	P.D. MILLI-VOLTS
	Original colloid			Electrodialyzed colloid		
0.0	0	-2.70	-56.7	0	-3.20	-67.2
0.5	0	-2.85	-58.8	0	-3.10	-65.1
1.0	0	-2.85	-58.8	x	-2.60	-54.6
4.0	0	-2.70	-56.7	xxxx	-2.40	-50.5
8.0	0	-2.58	-54.2	xxxx	-2.06	-43.3
12.0	xx	-2.40	-50.5	xxxx	-1.94	-40.7
16.0	xxx	-2.35	-49.3	xxxx	-1.81	-38.0
20.0	xxxx	-2.35	-49.3	xxxx	-1.76	-37.0
	Na-saturated			Ca-saturated		
0.0	0	-3.65	-76.6	0	-1.91	-40.1
0.5	0	-3.48	-73.1	0	-1.93	-40.5
1.0	0	-3.18	-66.7	0	-2.11	-44.3
4.0	0	-3.03	-63.7	0	-2.32	-48.7
8.0	0	-2.91	-61.2	0	-2.41	-50.6
12.0	xx	-2.61	-54.8	xx	-2.36	-49.5
16.0	xxx	-2.52	-53.0	xxx	-2.30	-48.3
20.0	xxxx	-2.58	-54.2	xxxx	-2.26	-47.5

TABLE 19
Influence of CaCl_2 and of $\text{Ca}(\text{OH})_2$ on the Sharkey colloid
 (0.4 gm. colloid per liter)

CaCl_2 MILLIEQUIVALENTS IN LITER	Ca-saturated			ELECTRODIALYZED		
	Flocculation	$\mu/\text{sec.}$ 1 volt/cm.	P.D. millivolts	Flocculation	$\mu/\text{sec.}$ 1 volt/cm.	P.D. millivolts
0.0	0	-1.91	-40.0	0	-3.20	-67.2
0.2	0	-1.40	-29.4
0.4	0	-1.22	-25.6	xx	-1.05	-22.0
0.5	xxx	-1.02	-21.4
0.7	xxxx	-0.89	-18.7
0.8	x	-1.06	-22.2
1.2	xxx	-1.03	-21.6
1.6	xxxx	-0.97	-20.4
$\text{Ca}(\text{OH})_2$						
0.0	0	-1.91	-40.0	0	-3.20	-67.2
0.4	0	-1.52	-31.9	0	-1.69	-35.5
0.6	x	-1.21	-25.4
0.8	xxx	-1.09	-22.9	0	-1.44	-30.2
1.0	xxxx	-1.02	-21.4	x	-1.13	-23.7
1.2	xxxx	-0.93	-19.5	xxx	-0.98	-20.6
1.4	xxxx	-0.95	-19.9

increase in concentration. It appears that the cataphoretic potential depends on the nature of the adsorbed cation, whereas the *suppression* of the P.D. and the stability are governed by the free electrolyte. All the suspensions in this experiment flocculate at about 50 millivolts, but in the case of the Ca-saturated colloid, as in the above case of bentonite, flocculation takes place at a considerably higher P.D. than that of the original suspension containing no chloride. Again we find no parallelism between the cataphoretic potential and the stability.

TABLE 20
Influence of $AlCl_3$ and of methylene blue on the Sharkey soil colloid
(0.2 gm. colloid per liter)

$AlCl_3$ MILLIEQUIVA- LENTS IN LITER	UNTREATED COLLOID			ELECTRODIALYZED COLLOID		
	Flocculation	μ /sec. 1 volt/cm.	P.D. millivolts	Flocculation	μ /sec. 1 volt/cm.	P.D. millivolts
0.0	0	-2.40	-50.5	0	-3.20	-67.2
0.1	x	-1.67	-35.0	xxx	-1.60	-33.6
0.2	xxxx	-1.62	-34.0	xxxx	-0.80	-16.8
0.4	xxxx	-0.47	-9.9	xxxx	-0.59	-12.4
0.7	xxxx	-0.18	-3.8	xxxx	-0.45	-9.4
1.0	xxxx	-slight	xxxx	-0.17	-3.6
1.5	xxxx	+0.48	+10.1	xxxx	± 0.0	± 0.0
2.0	xxxx	+0.61	+12.3	xxxx	+0.14	+2.9
4.0	xxxx	+0.78	+16.4	xxxx	+0.49	+10.3
Methylene blue						
0.032	0	-2.0	-42.0	x	-2.2	-46.2
0.043	0	-1.8	-37.8	xx	-2.0	-42.0
0.054	xx	-2.0	-42.0	xxx	-1.8	-37.8
0.064	xxx	-1.6	-33.6	xxxx	-1.1	-23.1
0.080	xxx	-1.4	-29.0	xxxx	-slight
0.107	xxxx	-1.3	-27.3	0	+1.4	+29.0
0.121	xxxx	-0.8	-16.8
0.134	xxxx	-slight
0.139	xxxx	+0.6	+12.6
0.145	xxx	+1.1	+23.1
0.161	0	+2.2	+46.2	()	+3.0	+63.0

Table 19 shows the effect of $CaCl_2$ and $Ca(OH)_2$ on the Ca-saturated and electro dialysed Sharkey soil colloid.

As in the case of KCl, the unsaturated colloid is flocculated in a considerable lower concentration of $CaCl_2$ than is the Ca-saturated colloid, which, as already stated, must be because the exchange acidity liberates Al and Fe ions, resulting in the formation of electropositive oxychlorides. The weaker flocculating power of the chloride in the Ca-saturated colloid is not due to a peptizing effect of $Ca(OH)_2$, which in the saturated colloid is seen to flocculate even more powerfully than the chloride, but to the absence of an exchange acidity, and

its secondary products. In the case of the hydroxide the relationship is reversed the unsaturated material requiring about 0.4 milliequivalent base per liter more than the colloid already saturated with Ca. Since one liter contained 0.4 gm. colloid the difference is roughly equal to the quantity of base required to saturate the colloid, which is about 0.8 milliequivalents per gram. Experiments with more concentrated suspensions showed that the quantities of Ca(OH)_2 required for flocculation increased progressively with the concentrations of the electrodialyzed material while the concentrations of the colloid already saturated with the base had very little influence on the concentration of CaCl_2 and Ca(OH)_2 required for flocculation.

It may be stated as a general rule that the colloid concentration influences greatly the flocculating value of any electrolyte which is adsorbed by the colloid while this is not the case when the electrolytes are not adsorbed. This again supports the view that it is the free electrolyte which is responsible for the suppression of the P. D. and stability as demanded by the Donnan equilibrium. The nature of the adsorbed cation determines the initial charge; it is only the suppression of this charge which is governed by the free electrolyte.

Comparing the critical potential in the last two tables we find that although KCl flocculated at about 50 millivolts the chloride and hydroxide of calcium do not flocculate until the P.D. is reduced to 20 millivolts.

Table 20 shows the influence of AlCl_3 and of methylene blue (tetramethylthionin chloride, $\text{C}_{16}\text{H}_{18}\text{N}_2\text{S}\text{Cl} + 3\text{H}_2\text{O}$, M. W. = 373.6) on the Sharkey colloid. In the case of the action of AlCl_3 , there is a slight difference in the flocculating values and a considerable difference between the isoelectric points of the untreated or saturated colloid and the electrodialyzed or unsaturated colloid. On the basis of the author's previous work (21) this is easily explained. It has been shown that the products of hydrolysis of aluminum salts, i.e., the oxychlorides which constitute an electropositive sol, are far more active in suppressing the charge and stability of electronegative colloids than are the nonhydrolyzed salts themselves. In 0.2 gm. of the untreated neutral colloid there is $0.2 \times 0.8 = 0.16$ milliequivalent displaceable bases. In the first two concentrations of AlCl_3 , or up to 0.2 milliequivalent, the chloride is rendered inactive by the formation of the normal hydroxide, which is not electropositive. The effect is here merely due to the chlorides of the displaced base (displaced by H and not by Al). The effect of the AlCl_3 , which is indirect in the saturated colloid, is therefore here weaker than in the case of the unsaturated colloid as far as the lowest concentrations are concerned. In higher concentrations the AlCl_3 is only partly neutralized by the bases in the saturated colloid, resulting in the formation of the highly electropositive oxychloride. It requires therefore less AlCl_3 to neutralize the negative charge of the saturated than that of the unsaturated colloid. In the latter suspension the aluminum salt is much less hydrolyzed.

An instructive experiment with the unsaturated colloid- AlCl_3 system may be performed. If AlCl_3 , insufficient electrically to neutralize the negative

colloid, is added and if the chloride is then partly alkalinized by the addition of NaOH, the floc becomes at once isoelectric or electropositive. We are confronted therefore with the paradox of seeing NaOH render a negative colloid isoelectric or even electropositive but if an excess of NaOH is added this base will act true to form and charge the colloid strongly electronegative and disperse it. The explanation is that in the partial alkalization we are chemically producing an electropositive sol whereas with an excess of alkali we destroy the electropositive behavior by forming the normal hydroxide of aluminum, which, like all other materials, is electronegative in NaOH. These facts are of the greatest importance for the process of water purification and in the reclamation of alkali soils in which these agents are employed.

The action of methylene blue on soil colloid has been discussed in two previous papers (16, 21). This cation is completely adsorbed over the entire range on the electronegative side of the isoelectric point. The quantity of the dye required to render the colloid isoelectric is therefore directly proportional to the quantity of colloid. In the case of neutral soil colloids, and in the absence of other electrolytes, this quantity of the dye was found to be equivalent to the quantities of exchangeable bases, so that, in the case of different colloids, the quantities of methylene blue required to neutralize their negative charge is a direct measure of their exchange capacity. The methylene blue cation, which actively displaces the common cations, does not seem to be dissociated by the particles. When all the common cations have been displaced the colloid therefore will be isoelectric. This explains the equivalence. The adsorption proceeds beyond the isoelectric point but becomes more and more incomplete with an increase in the positive charge. In the electropositive condition the anion, i.e., the Cl ion, must make up the micellar ion atmosphere.

It will be seen that the unsaturated colloid requires less methylene blue both for flocculation and for electrical neutralization. This is, as already explained, due to the exchange acidity, which here attains a maximum because of the great activity of this cation. Furthermore the exchange capacity of the colloid has been shown to decrease with the pH (19). The addition of electrolytes to the methylene blue-soil colloid system has the following effect: Bases and salts with di- and poly-valent anions cause a greater quantity of the dye to be required for the electrical neutralization of the colloid. Acids and salts with di- and tri-valent cations have the opposite effect.

The critical P.D. in the preceding experiment we find to be about 30 millivolts.

Table 21 shows the action of the NaOH on the electrodyalyzed and Ca-saturated Sharkey colloid. As is well known, the NaOH flocculates only in comparatively high concentrations but the Ca-saturated colloid is more sensitive than the electrodyalyzed, or rather the Na-saturated, colloid. (The unsaturated colloid is of course immediately saturated with the added base.) The anomaly of the critical potential is here very great. The Na-saturated colloid flocculates

at as high a P.D. as 64 millivolts and the Ca-saturated at 56, which is again higher than the P.D. of the original stable suspension.

The preceding experiments show the influence of individual electrolytes on the stability and cataphoresis of the colloids. Since in the soil the action is always due to a combination of electrolytes, it will be of interest to study the effect of a few such combinations. Table 22 and 23 show the influence of the presence of actively charging electrolytes, such as NaOH and $\text{Na}_4\text{Fe}(\text{CN})_6$ on the flocculating, and the discharging action of $\text{Ca}(\text{OH})_2$, of CaCl_2 , and of methylene blue. The tables should be compared with table 19 and 20, which show the action of the same electrolytes on the Sharkey soil colloid without the

TABLE 21
The influence of NaOH on electrodialysed and Ca-saturated Sharkey colloid
(0.4 gm. colloid per liter)

NaOH MILLIEQUIVALENTS IN LITER	FLOCCULATION	$\mu/\text{SEC.}$ 1 VOLT/CM.	P.D. MILLIVOLTS
<i>Electrodialysed colloid</i>			
0.0	0	-3.25	-68.2
0.2	0	-3.60	-75.6
1.0	0	-3.93	-82.5
4.0	0	-3.70	-77.7
8.0	0	-3.52	-74.0
12.0	0	-3.45	-72.5
16.0	0	-3.18	-66.8
20.0	xx	-3.08	-64.7
24.0	xxxx	-3.08	-64.7
28.0	xxxx	-3.05	-64.0
<i>Ca-saturated colloid</i>			
0.0	0	-1.91	-40.0
0.2	0	-3.06	-64.2
1.0	0	-3.39	-71.1
4.0	0	-3.04	-63.8
8.0	x	-2.81	-59.0
12.0	xxxx	-2.68	-56.3

hydroxide and ferrocyanide of sodium. The comparison shows that the NaOH in quantities far below its own flocculating concentration, materially reduces the amount of CaCl_2 and $\text{Ca}(\text{OH})_2$ required to flocculate the *saturated* colloid, in spite of the fact that the alkali hydroxide greatly increases the cataphoretic potential. The critical potential is more than doubled by the presence of NaOH. This is in exact agreement with the author's observations made several years ago in the case of suspensions of quartz, clay, and humus (15). The comparison must only be made with the saturated colloids. The unsaturated colloid is more sensitive to the neutral salts alone, for the reasons explained. The suppressing effect of hydroxides on the flocculating power of salts, which

TABLE 22

The influence of NaOH on the action of CaCl_2 and of $\text{Ca}(\text{OH})_2$ on the Na-saturated Sharkey colloid

(0.4 gm. colloid per liter)

CaCl_2 MILLIEQUIVALENTS IN LITER	NaOH MILLIEQUIVALENTS IN LITER	FLOCCULATION	$\mu/\text{SEC.}$ 1 VOLT/CM.	P.D. MILLIVOLTS
0.0	2.0	0	-3.86	-81.0
0.4	2.0	0	-3.01	-63.2
0.6	2.0	xx	-2.42	-51.8
0.8	2.0	xxxx	-2.13	-44.7
1.0	2.0	xxxx	-1.58	-33.2
$\text{Ca}(\text{OH})_2$				
0.0	2.0	0	-3.88	-81.5
0.4	2.0	0	-3.08	-64.7
0.6	2.0	xxxx	-2.49	-52.3
0.8	2.0	xxxx	-1.95	-41.0
1.0	2.0	xxxx	-1.50	-31.5

TABLE 23

The influence of $\text{Na}_4\text{Fe}(\text{CN})_6$ on the action of CaCl_2 and of methylene blue on the Na-saturated Sharkey colloid

(0.2 gm. colloid per liter)

CaCl_2 MILLIEQUIVALENTS IN LITER	$\text{Na}_4\text{Fe}(\text{CN})_6$ MILLIEQUIVALENTS IN LITER	FLOCCULATION	$\mu/\text{SEC.}$ 1 VOLT/CM.	P.D. MILLIVOLTS
0.0	2.0	0	-3.92	-82.3
0.4	2.0	0	-3.11	-65.3
0.8	2.0	0	-2.55	-53.6
1.0	2.0	0	-2.44	-51.2
1.2	2.0	0	-2.12	-44.5
1.6	2.0	xx	-1.86	-39.1
2.0	2.0	xxxx	-1.73	-36.3
2.4	2.0	xxxx	-1.66	-34.8
Methylene blue				
0	1.5	0	-4.05	-85.0
0.080	1.5	xxxx	-3.20	-67.2
0.107	1.5	xxxx	-3.20	-67.2
0.161	1.5	xxxx	-2.15	-65.8
0.268	1.5	xxxx	-1.05	-22.1
0.537	1.5	xxxx	-0.28	-5.9
0.805	1.5	xxxx	± 0.0	± 0.0

has often been observed, is evidently not due to the presence of the base, as assumed, but to the absence of the more powerfully acting products of the exchange acidity.

The presence of the ferrocyanide caused a slight suppression in the flocculat-

ing power of CaCl_2 , whereas the critical potential was nearly doubled. In the case of the methylene blue, its flocculating power was at least not suppressed by the presence of the tetravalent anion but the tetravalent anion more than doubled the critical potential. The fact, previously alluded to, that the presence of polyvalent anions causes an increase in the quantity of methylene blue required to neutralize the negative charge of the colloid, is here strikingly illustrated. About six times more methylene blue was required for the electrical neutralization in the presence of the tetravalent anion. The same effect of this ion was observed in the preparation of isoelectric alumino-silicates. The proportion of electropositive alumina required to neutralize electrically the negative silica was greatly increased by the presence of a small quantity of ferrocyanide (20). This phenomenon is being investigated in connection with a study of various isoelectric systems and will be discussed in a later publication.

THE EFFECTS OF ACIDS

Negative and Positive Soil Colloids

Because of the amphoteric behavior of soil colloids which contain a high proportion of sesquioxides, the influence of acids may be very different in different colloids, depending upon the composition of the colloids. The author has already shown how cataphoresis in acid solutions is related to the silica/sesquioxide ratio (19). It has also been shown that those colloids which are charged electropositively in acid solutions adsorb appreciable quantities of the anions of the acid. The order of magnitude of the adsorption is:



Of these ions only the phosphate is adsorbed from neutral and alkaline solutions but the adsorption is much greater in acid solutions. The adsorption of all the anions mentioned, increases progressively with an increase of sesquioxide content of the colloid. The relationship between the positive charge and the anion adsorption was explained by assuming the formation of compounds like oxychlorides, which dissociate into diffusible anions and an electropositive colloidal complex.

It was found that the magnitude of the positive charge bore no relation to the quantity of anions adsorbed, which was always greatest in the case of the PO_4 ion and least in the case of the Cl . The charge was very much greater in the HCl than in the H_2SO_4 solution. The H_3PO_4 , which at low pH values dissociates only one H, acted more like HCl . The influence of these acids upon the amphoteric soil colloids therefore resembles closely their influence upon the proteins, as observed by Loeb. It is evident that the charge depends to a great extent upon the valence.

The influence of the aforementioned three acids on the cataphoresis of two colloids very different in composition is shown in tables 24 and 25. The Mar-

shall silt loam colloid, which has a silica/sesquioxide ratio of 2.82, is strictly electronegative, whereas the Aragon clay subsoil colloid, with a ratio of 0.55, is amphoteric in behavior (19). Since, in the case of the Aragon colloid,

TABLE 24
Cataphoresis of the Marshall soil colloid in HCl, H₂SO₄, and H₃PO₄ solutions
(0.2 gm. colloid per liter)

MILLIEQUIVALENTS IN LITER	HCl		H ₂ SO ₄		H ₃ PO ₄	
	μ /sec. 1 volt/cm.	P.D. millivolts	μ /sec. 1 volt/cm.	P.D. millivolts	μ /sec. 1 volt/cm.	P.D. millivolts
0.0	-2.35	-48.3
0.1	-1.85	-38.8	-2.05	-43.0	-1.91	-40.0
0.4	-1.83	-38.4	-2.20	-46.2	-1.76	-37.0
1.0	-1.75	-36.8	-2.25	-47.2	-1.63	-34.2
4.0	-1.55	-32.5	-1.91	-40.0	-1.65	-34.6

TABLE 25
Cataphoresis of the Aragon soil colloid in HCl, H₂SO₄, and H₃PO₄ solutions
(0.2 gm. colloid per liter)

MILLIEQUIVALENTS IN LITER	pH	FLOCCULATION	μ /SEC. 1 VOLT/CM.	P.D. MILLIVOLTS
HCl				
0	XX	-1.07	-22.5
0.01	6.10	XXXX	+0.32	+6.7
0.1	4.53	XXXX	+1.58	+33.2
1.0	3.25	XXXX	+1.90	+39.9
4.0	2.55	XXXX	+2.18	+45.8
H ₂ SO ₄				
0.01	6.25	XXXX	+0.10	+2.1
0.1	4.40	XXXX	+0.73	+15.3
1.0	3.25	XXXX	+0.39	+8.2
4.0	2.59	XXXX	+0.23	+4.8
H ₃ PO ₄				
0.03	6.05	0	-1.26	-26.4
0.3	4.47	XXXX	± 0.0	± 0.0
3.0	3.28	XXXX	+0.68	+14.3
12.0	2.70	XXXX	+0.63	+13.2

adsorption of the acids was considerable, the pH values were determined by the quinhydrone electrode. This was done after three days standing.

In the case of the Marshall soil colloid there is no great reduction in the negative charge, and the suppressing effect of the acids would undoubtedly be even smaller if it were not for the formation of secondary products. Quartz

particles increase their speed of migration in a dilute HCl solution. The valence effect of the anions is unmistakable and it is interesting to note that the phosphoric acid suppresses the charge fully as much as the hydrochloric, whereas the sulfuric acid alone exerts a weaker suppression. This is, as pointed out by Loeb, because free H_3PO_4 dissociates chiefly the monovalent anion H_2PO_4^- . In neutral sodium phosphate the charge of the particles is considerably increased. The maximum speed of migration of the Marshall particles in solutions of KCl, K_2SO_4 , and neutral sodium phosphate was found to be 2.8, 3.0, and 4.0 μ per second respectively.

The effect of the three acids on the Aragon colloid is very different. This colloid, which has a very high sesquioxide content, is charged electropositively in a carbonic acid solution. In freshly distilled water the particles migrated toward the anode with an average velocity of 1.07 μ per second. In old CO_2 -contaminated water the material was about isoelectric. A concentration of 0.01 milliequivalent of HCl and H_2SO_4 per liter was sufficient to change the sign of charge but, whereas the positive charge is progressively increased by the HCl over the entire concentration range, it soon reaches a maximum in the H_2SO_4 solution beyond which the positive charge is suppressed by the free, divalent anion. The valence effect on the positive side acts in the opposite direction to that on the negative side of the isoelectric point, the divalent anion causing the greatest suppression of the positive charge.

The effect of the H_3PO_4 shows an interesting deviation. The lowest concentration (0.01 millimol) charges the particles more electronegatively and brings about a decided peptisation, as was shown by lasting turbidity in this case whereas all the other acidified suspensions settled clear over night. Even the water suspension flocculated about half completely. (The experiment was repeated with the same results.) The next higher H_3PO_4 concentration rendered the colloid isoelectric, whereas the two highest concentrations rendered the particles more strongly electropositive than in the case of the corresponding H_2SO_4 concentrations. This is evidently because the monovalent di-hydrogen phosphate ion suppresses the positive charge less strongly than does the divalent SO_4 ion.

The initial elevation of the negative charge by the H_3PO_4 might be assumed to be due to the formation of phosphates yielding divalent and possibly trivalent anions. But just why these ions should increase the cataphoretic potential is, in view of the above observations, not so easy to explain. The phosphate ion is adsorbed by all soil colloids under all conditions of reaction and, according to the generally accepted view, an adsorbed anion should increase the negative charge. But the alkali sulfates, and even more strongly the ferrocyanides, increase the negative charge of negative colloids, and yet these anions are apparently not positively adsorbed, but are, on the contrary, negatively adsorbed because of Donnan distribution. In acid solutions, the Cl and the SO_4 ions are both adsorbed to an appreciable extent by soil colloids with a high sesquioxide content. But this adsorption of the anions, instead of

increasing the negative charge, brings about its destruction and imparts a positive charge to the colloid. The magnitude of this positive charge bears however, no relation to the quantity adsorbed. The Cl ion which is least adsorbed imparts the strongest positive charge.

This relationship between the anion adsorption and the positive charge is satisfactorily accounted for by the formation of basic salt complexes by an interaction between the sesquioxides and the acids. A dissociation of these combinations results in an atmosphere of anions surrounding an electropositive particle just as the electronegative particle is surrounded by an atmosphere of cations. The magnitude of the charge must depend, among other factors to be mentioned later, on the degree of dissociation, i.e., on the number of free charges per unit surface. It is in this connection that the specific nature of the ions plays an important rôle and gives rise to the Hofmeister or lyotropic ion series.

On the basis of this theory the increase in charge resulting from the addition of certain electrolytes might be explained. If the micellar ion is different from the corresponding ion in the added electrolyte an exchange is known to take place. If the ingoing ion remains dissociated to a greater extent than the outgoing ion an increase in charge must follow, provided that the suppressing effect of the free electrolyte is not great enough to counteract the increase, as will always be the case in higher concentrations. If we look at table 18 we will find that the KCl increased the charge in low concentrations only in the case of the original (chiefly Ca- and Mg-saturated) and the Ca-saturated colloids but not in the case of the H- and Na-saturated colloids. In table 21 we see that NaOH increased the charge in the case of the H-saturated as well as in the case of the Ca-saturated colloid. In other words the charge, as measured by cataphoresis, is increased whenever the displacing ion gives rise to a more highly dissociated complex. When the displacing ion is of the more highly associating type such as the divalent ions and the methylene blue ion, then the charge is reduced at the outset. (The aforementioned anomaly of bentonite will be discussed later.)

Effect of salts on positive colloids

Before any work had been done on the negative adsorption or Donnan distribution in the case of electropositive soil colloid gels, it was concluded from the cataphoresis experiment with the Aragon colloid in acid solutions that the Cl ion is more highly dissociated by the particles than is the SO_4 ion. If this were the case then the addition of a chloride to the sulfated complex of the colloid should increase the positive charge just as the addition of an alkali salt increases the negative charge of the Ca-saturated colloid. It has already been pointed out that the adsorbed anions displace one another (19). But since the different anions are "adsorbed" in very different proportions their exchange differs radically from base exchange which takes place in equivalent proportions. And just as an alkali sulfate imparts a stronger negative charge than an alkali chloride to the electronegative Ca-saturated colloid because of the weaker

suppressing influence of the divalent anion of the free electrolyte, so, for the same reasons, but in a reversed sense, must a chloride with a divalent cation permit a greater increase in the positive charge of the electropositive SO_4 -complex. On the other hand, the addition of sulfates and, even more so, the addition of ferrocyanides must suppress the positive charge at the outset.

The experiment, shown in table 26, fully confirmed the predictions.

The chlorides did not greatly increase the positive charge, but it must be remembered that an increase due to the formation of a more highly ionized Cl -complex is opposed at the outset by the suppressing influence of the free electrolyte. The suppression is greatest in the case of NaCl and weakest in the case of CaCl_2 because the ion of the same sign of charge as the colloid, i.e., the cation, is divalent in the latter salt. (Compare table 2, part I.)

TABLE 26
Influence of salts on the electro-positive SO_4 -complex of the Aragon colloid
(0.2 milliequivalents H_2SO_4 and 0.2 gm. colloid per liter)

SALT MILLIEQUIVALENTS IN LITER	FLOCCULATION	$\mu/\text{SEC.}$ 1 VOLT/CM.	P.D. MILLIVOLTS
0	XXXX	+0.89	+18.7
NaCl			
0.2	XXXX	+0.94	+19.7
1.0	XXXX	+1.05	+22.1
CaCl_2			
0.2	XXXX	+1.08	+22.7
1.0	XXXX	+1.16	+24.4
Na_2SO_4			
0.2	XXXX	+0.72	+15.1
1.0	XXXX	+0.42	+8.8
$\text{Na}_4\text{Fe}(\text{CN})_6$			
0.2	XX	-0.64	-13.4
1.0	X	-1.89	-39.7

The sulfate exerts only a suppressing effect on the charge of the sulfated complex. This is similar to the action of CaCl_2 on the Ca -saturated complex.

The ferrocyanide not only suppresses the positive charge but renders the colloid electronegative. This is to some slight extent due to a suppression of the hydrogen-ion concentration, but the pH was appreciably displaced only in the higher ferrocyanide concentration. It was low enough (about 4.5) even here however, to impart a positive charge in the absence of the salt. As far as the phenomenon may be related to the Donnan equilibrium the effect can only be in the form of a suppression. The suppression must in this case be very great because of the four valences of the anion. But the Donnan distribution of the free electrolyte can only exert a discharging effect; it cannot increase the charge nor change its sign. We may conclude that the di- and trivalent phosphate

ions and the tetravalent ferrocyanide ion form a nondissociated complex with the soil material. The particles must then assume a negative charge by the same mechanism by which all other materials charge themselves electronegatively in aqueous suspension. The electropositive charge is apparently confined to a special condition of matter, by virtue of which diffusible anions are dissociated by the dispersed phase. The electronegative behavior of materials dispersed in water appears to represent a more general condition. A conception of how this may be related to the structure and properties of the interfacial layer of water has been presented in previous papers (20, 21).

Regarding the stability of the Aragon colloid we find again no definite relationship to the cataphoretic potential. While the lowest H_3PO_4 concentration and the ferrocyanide caused a distinct peptisation, all the other suspensions were completely flocculated although some of them showed a higher potential or, to speak more cautiously, a higher migration velocity.

It should be pointed out that the migration velocity may not be directly proportional to the cataphoretic potential, as would be the case if all the other factors in the Helmholtz-Perrin formula remain constant. But we are hardly justified in assuming values for the viscosity and the dielectric constant equal to that of ordinary "free" water. The water molecules or hydrols within the micellar atmosphere must, insofar as they are electrical bipoles, occupy an oriented position. This means that "adsorbed" water is structurally different from "free" water. The oriented hydrols are under the influence of an electrical moment and therefore must offer a greater resistance to a displacement, as in cataphoresis, than would be offered by free hydrols distributed at random. A greater viscosity within the double layer may therefore be assumed. The addition of electrolytes may diminish, in various degrees, the electrical moment and the orientation, resulting in a decreased viscosity. Electrolytes are known to diminish the dielectric constant. It is therefore evident that there can be no exact proportionality between cataphoresis and the P.D. of the double layer. Nevertheless, the migration velocities at the point of flocculation differ so greatly (and are in certain well-defined cases even greater at this point than before the electrolyte is added) as to make it very doubtful that flocculation takes place at the same P.D. in every case. In the case of colloid, oil drops, and other inert materials the critical potential may be very nearly the same for all electrolytes, but in the case of soil particles which are surrounded by a dense atmosphere of ions and a comparatively thick layer of osmotically imbibed water, the critical potential seems to vary with the nature of the ions. This indicates the existence of other stability factors.

FACTORS OF STABILITY

Dissociation

The idea that flocculation is caused by an adsorption of the ions of opposite sign of charge which neutralize the free charges on the surface of the particles

and thereby suppress the cataphoretic potential, does not provide us with an adequate explanation. It has been shown that the ions of the same sign of charge as the colloid influence flocculation according to their valence and that this is true even when there is no adsorption of the ions. Given (9) and later Mattson (15) found the same influence in the case of different calcium salts, and it was significantly observed by P. Ehrenberg (7, p. 303) that the SO_4 ion was not adsorbed by the clay although it had been found that the ion did influence the flocculation. If the observation made in part I of this paper, that the dissociation of the colloid complex, i.e., the value of z , is increased by the addition of electrolytes, is substantiated, then, neither discharge nor flocculation can be the result of adsorption or a suppression of ionization of the ion of opposite sign of charge, and our theory will call for a radical revision.

An increase in dissociation of the complex with an increase in the added electrolyte is not so absurd as it might at first seem. If the base is adsorbed by virtue of a fixation of the OH ions at the interface then the cations are in reality not by themselves adsorbed but are merely attracted by the ions of opposite sign at the interface. Only a few of the cations are able to diffuse into the external medium because a critical potential is established against which the ions cannot dissociate. This limiting potential appears to be equal to that of the common ions, or about 70 millivolts. As far as formula (C) (see part I) is applicable, the author (17) calculated that in the case of a colloidal particle with a radius of 45.5μ and containing 505,208 exchangeable cations, a dissociation of only 857 ions would account for the cataphoretic potential. In the absence of other electrolytes a very slight dissociation is apparently sufficient to raise the potential to the limiting value. The addition of electrolytes suppresses the potential, thus weakening the electrostatic attraction on the cations and causing a greater number of them to diffuse into the outer medium. This explains the constancy of the product (P.D.) z which seems to prove the connection between dissociation and the potential. Thus by looking upon the colloidal complex as an adsorption ionogen which holds one of the ions (that of opposite charge) merely to preserve a certain electrical balance, it becomes comprehensible why a suppression of the P.D. leads to a greater dissociation.

Activity

The preceding phenomenon is similar to the "salt effect" on weak electrolytes as observed in connection with the indicator method and described by Brönsted (1a) as an expression of a change in the activity or potential of the ions. The interionic attraction theory of activity as formulated by Debye and Hückel (5) to explain the deviations of strong electrolytes from the ideal gas laws has much in common with our conception of the colloidal micelle and might successfully be applied to the problem here dealt with. This theory takes into account the mutual attraction between the ions of opposite charge. This attraction leads to the condition that in the neighborhood of an ion there is on the average more ions of unlike than of like sign of charge. The result

of this interionic action is a suppression of the potential, or activity, of the individual ions. When two ions of unlike sign are a great distance apart, as in very dilute solutions, their potential or activity approaches a maximum. As two such ions are brought closer together, their potentials are progressively suppressed as their fields overlap. An actual contact or association of the ions results in a zero potential, except in so far as a residual field remains, because of a polarization from an asymmetry in the molecule. Since the Coulomb forces between the ions are inversely proportional to the square of the distance between them, it follows that the suppression of the self-potential or activity of the ions must be proportional to the square root of the concentration. Thus the molar conductivities of strong electrolytes decrease with an increase in concentration according to the well-known Kohlrausch's square-root law.

This, in common language, may serve to illustrate the fundamental concept in the interionic attraction theory. The mathematical formulation of the theory has led to the following simple limiting equation applicable to dilute solutions

$$-\log \gamma_s = 0.5 Z_a Z_b \sqrt{\mu}$$

where γ_s is the mean activity coefficient of the ions a and b of a compound, Z_a and Z_b the valence of the ions, and μ the ionic strength of the solution. $\mu = \frac{1}{2} \sum m_i z_i^2 = \frac{1}{2} \sum c_i z_i^2$ where m is molality, c equivalent concentration, and z valence of any ion present in the solution (13).

Thus if in a solution of a weak acid HA , μ is increased by the addition of a salt, then the activity coefficient of the ions will be diminished in proportion to $\sqrt{\mu}$ while the activity coefficient of the neutral molecules will be unaffected. This leads to an increase in dissociation in accordance to the expression

$$\frac{\gamma_{H^+} \gamma_{A^-}}{\gamma_{HA}} = K_\gamma$$

in which K_γ remains a true constant which would of course not be the case in the classical mass action expression

$$\frac{C_{H^+} C_{A^-}}{C_{HA}} = K_c$$

in which the factors represent concentration instead of activity.

Similarly in the case of a slightly soluble salt an increase in μ by the addition of an extraneous salt will result in a greater solubility as the activity coefficient of the ions is diminished. In the presence of the solid phase the concentration of the dissolved salt multiplied by the activity coefficient should remain constant, thus

$$[\text{salt}]_1, \gamma_1 = [\text{salt}]_2 \gamma_2 \text{ etc.}$$

where the brackets signify concentration.

By introducing the above equation we get

$$\log \frac{[\text{salt}]_1}{[\text{salt}]_2} = \log \gamma_2 - \log \gamma_1 = 0.5 Z_a Z_b (\sqrt{\mu_1} - \sqrt{\mu_2})$$

which shows the logarithm of the ratio of the two solubilities of the salt to be a linear function of the square root of the ionic strength of the solution. For applications of this theory the reader is referred to Börnsted and LaMer (2, 29), Noyes (24), Müller (23), and to Clark (4, chap. XXV).

The foregoing illustrates the operation of the square root law as far as this factor alone affects the activities of the ions of a given compound under study. In the preceding equation there is another factor which, for the problem here dealt with, is of fundamental importance and deserves attention. This is the influence of the valence of the ions of the compound affected, which is expressed by the valence factor $Z_a Z_b$. From this it is evident that the suppression of the activity coefficient will increase with the valence of the ions of the compound, the suppression being proportional to the product of the valence of the two ions. The coefficient $0.5 Z_a Z_b = 0.5$ for a uni-univalent salt; for a uni-bivalent it is $= 1$; for a uni-trivalent it is $= 1.5$; for a tri-trivalent it is $= 4.5$. Now it becomes at once evident that, as far as the equation is at all applicable to colloidal ionogens, one of the ions of which is greatly multivalent, the suppressing effect of an added salt on the activity or potential of the ions will be very much greater as compared to the effect on ordinary electrolytes. This will explain why the value of z , i.e., the concentration in the micellar atmosphere of the ions dissociated by the colloid, was found to increase so rapidly with an increase in the amount of salt added. The value of z increased nearly ten-fold between a salt concentration of $0.005 N$ to $0.4 N$ (see table 3).

Dissociation and ionic strength

In view of what has been said in the foregoing, the significance of a relationship, not before mentioned, will readily be recognized. If the z values as ordinates are plotted against the square roots of the corresponding y concentrations, as in figure 4, a straight line is obtained which, when extrapolated, passes through a point very near the origin. The values are taken from tables 3 and 7, part I. Here \sqrt{y} is equal to $\sqrt{\mu}$, since the added salt (NaCl) is uni-univalent.

The linear relation shows: first, a connection between the dissociation of the colloid and the square root of the ionic strength; and, second, that the colloid is only very slightly dissociated in the absence of an extraneous electrolyte.

It follows from purely electrokinetic considerations that a highly multivalent colloidal complex cannot dissociate to the same extent as common salts. The latter dissociate into ions with only one, two, or three, electronic charges. The potential and the resulting attraction between these ions are never greater than can be overcome by the force of thermal agitation and the tendency to disperse. In the case of very small ions in which the self potential would be too high the

stability of the ions is increased by an association with the molecules of the solvent. Common salts may therefore be completely dissociated.

A colloidal ionogen, on the other hand, can only dissociate to a point at which a limiting potential is established. At this point the force of thermal agitation is balanced by the electrostatic attraction. In the absence of extraneous electrolytes, when the activity coefficient is at a maximum this condition is apparently attained at a comparatively low degree of dissociation. If a soil colloidal particle with a radius of $45.5 \mu\mu$ and containing 505,208 exchangeable cations were completely dissociated, the ion density would be extremely great, resulting

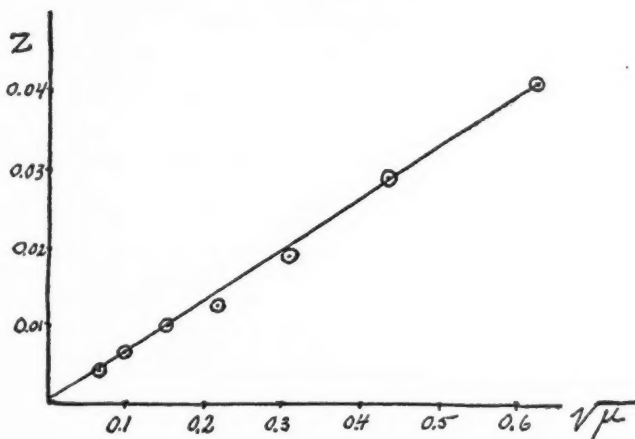


FIG. 4. THE LINEAR RELATION BETWEEN THE DISSOCIATION OF THE COLLOIDAL COMPLEX (VALUES OF Z) AND THE SQUARE ROOT OF THE IONIC STRENGTH OF THE SOLUTION

in a potential several hundred times as great as has ever been observed. Applying equation (C) part I,

$$\text{P.D.} = \frac{e \delta}{Dr (r + \delta)}$$

and assuming a mean thickness of the double layer $\delta = 5 \mu\mu = 5 \times 10^{-7}$ cm. yields a potential of 19.6 volts. This is nearly 300 times as great as the usually found maximum of 70 millivolts. It is obvious that a potential of this order of magnitude would powerfully attract the dissociated ions, leading to an association with the interfacial layer of ions of opposite sign of charge. The assumed thickness of the double layer of $5 \mu\mu$ was certainly taken low enough in view of the preceding observed thickness of the ion atmosphere (see part I) but even if we assume a thickness of molecular dimensions, which would be absurd, the calculated potential would still be much too high.

Since the value of z , or the number of free charges per unit surface, increases with the square root of the ionic strength, it is obvious that equation (C) cannot be applied in its present form in the presence of free electrolytes any more than the ordinary mass action law can be applied. In this equation a constant value for each electronic charge e is assumed but this must vary with the activity coefficient of the ions. For values smaller than unity the latter should necessarily be introduced in the equation thus

$$\text{P.D.} = \frac{e \gamma \vartheta}{Dr (\gamma + \vartheta)}$$

Dissociation of proteins

In his study of the proteins, Loeb (14) assumed a complete dissociation of the protein hydrochloride combinations. A careful examination of Loeb's data

TABLE 27
Percentage of dissociation of gelatin chloride calculated from Loeb's tables

M.EQ. HCl ADDED TO 100 CC. GELATIN SOLUTION	M.EQ. HCl ADDED TO 350 CC. OUTSIDE SOLUTION	TOTAL M.EQ. HCl ADDED	Cl ACCOUNTED FOR BY $x+y+z$ M.EQ.	DIFFERENCE = NONDISSOCI- ATED Cl M.EQ.	DISSOCIATED = z M.EQ.	PER CENT DISSOCIATION
0.1	0.0095†	0.1095	0.0448	0.0647	0.0165	20.3
0.2	0.0221*	0.2221	0.1123	0.1098	0.0514	31.9
0.6	0.136*	0.736	0.4061	0.3299	0.200	37.8
0.8	0.298*	1.098	0.8618	0.2362	0.343	59.3
1.0	0.581*	1.581	0.9702	0.6108	0.372	38.0
1.5	1.79*	3.29	2.740	0.55	0.608	52.6
2.0	3.04*	5.04	4.850	0.154	0.609	80.0

* Calculated from the pH values in column 4 table 1. All the other figures in this table are calculated directly from the corresponding values in tables 11 and 14 in the work referred to.

† Based on a pH value = y . A pH value = x would give 17.0 per cent dissociation. The true percentage dissociation should therefore be between 17.0 and 20.3 per cent.

reveals that this is not the case as far as the value of z , as calculated from the equilibrium equation, can be relied upon as an index of dissociation.

Loeb added HCl in increasing quantities to 100-cc. 1 per cent solutions of originally isoelectric gelatin contained in collodion bags, and immersed these bags in 350-cc. HCl solutions brought to the same pH as that of the gelatin solution inside the bags. By adding together the values of x , y , and z in terms of total milliequivalents in the outside and in side solutions at equilibrium and subtracting this sum from the total HCl added (a) to the 100-cc. gelatin solution and (b) to the 350-cc. outside solution, a difference was obtained for each concentration and, what is still more significant, the differences are relatively greater in low than in high concentrations. This means that the protein chloride is less dissociated in dilute than in more concentrated HCl solutions. This is contrary to the idea of a suppression of ionization by the

presence of a common ion but it is in harmony with the activity concept as applied, in the foregoing, to multivalent complexes.

The results of this calculation are given in table 27, the data being obtained from Loeb's tables 14, 11, and 1.

It will be seen that the calculated dissociation increases from about 20 to 80 per cent as the concentration of the free acid is increased. The increase, however, is, for some reason irregular. In the case of bentonite the dissociation increased from about 5 to 25 per cent as the concentration of NaCl was increased from 0.005 *N* to 0.4 *N*. The gelatin chloride is therefore more highly dissociated, although the Donnan P.D. is very nearly the same in the case of both materials at the same free electrolyte concentration. (Compare, for example, the action of NaNO₃ in Loeb's table 18 with the author's table 4 in part I.) The maximum quantity of HCl in combination with the gelatin is approximately the same as the quantity of base in combination with the saturated bentonite. The gelatin complex is therefore absolutely, as well as relatively, more dissociated than the bentonite complex. The nearly identical P.D. values, in spite of this difference in dissociation, are, of course, explained by the different concentrations used, i.e., a 1 per cent gelatin "solution" as compared to a 10 per cent bentonite gel.

The fact that the gelatin complex can dissociate to a greater extent than the bentonite complex without exceeding the critical potential must be due to the greater dispersion and especially to the greater hydration of the gelatin complex. A greater dispersion reduces the ion density to the unit surface, thus permitting a higher degree of dissociation. The probable effect of an extensive association with the water molecules must be a suppression of the potential, because these molecules, if polarized, are not neutral, and the cause of the association must itself depend upon the attraction between poles of unlike sign. Association with the water will therefore permit the complex to dissociate more ions. This is merely an application of a principle known to exist in the case of the common ions. Thus the self potential or activity of ions like Li or Na is suppressed by association with water molecules. We have already seen how a dehydration, e.g., by the addition of alcohol, increases the activity of these ions, which increase shows itself in a lower dissociation of the colloid complex (compare table 11). Further, it is well known that although the activity coefficient decreases in low to moderate concentrations, it again increases rapidly in high concentrations, and that the higher the hydration value of the ions is the greater is the augmenting effect in high concentrations (27). Thus the addition of large quantities of LiCl to a solution will have a greater effect upon the activity of the ions in solution than an equal quantity of NaCl or KCl because the Li ions, being more highly hydrated, compete more strongly for the water. This explains also why, in very strong solutions of the various chlorides, the difference in displacing power between the cations tends to vanish.

An increase in the dissociation of gelatin chloride with an increase in the concentration of the free electrolyte is also evident in the experiments of Loeb

where he studied the influence of neutral salts. These experiments, in which the HCl concentration was kept constant, are more comparable to the preceding experiments with the soil colloids, but, since Loeb studied the ion distribution by measuring the pH and does not give any data on the distribution of the ions of the salt, which is necessary for the calculation of z , the relationship between dissociation and the ionic strength is not so apparent. But a test calculation will show that if z remained the same, "neglecting the diminution of ionization of gelatin chloride" (14, p. 202), the suppressing effect of the salt would be much greater than it was found to be. In the experiment with NaNO_3 (14, table 18) the value of z equals 0.00275 N in the absence of salt. In the presence of .051625 M NaNO_3 , z must be equal to 0.0060 N to account for the reported P.D. value. In other words the dissociation has increased instead of diminished by the addition of the salt.

The increase in concentration weakens the activity of the ions and thus reduces the energy content of the solution. In view of the fact that the equilibrium between the undissociated complex and its ions must be an energy equilibrium rather than a mass equilibrium, it is easily understood that this effect of the salt will be a displacement of the equilibrium in the direction of increased dissociation. Thus the potential of a colloid particle or of a gel, being suppressed by the addition of an electrolyte, strives to maintain itself. This, within certain limits, is possible through a further dissociation. We may speak, therefore, of the colloid as being electrically buffered.

It thus appears that the observed increase in dissociation of the colloidal complex following an increase in salt concentration can be related to theoretically well-founded principles. The same phenomenon is encountered in the case of the simple ionogens but there is a quantitative difference between the two groups in that the effect is greatly intensified in the case of the colloid ionogen because of its highly multivalent character.

Present views on charge and adsorption

The generally accepted idea that the suppression of the interfacial potential and of the stability is due to a neutralization of the free charges by the adsorption of ions of unlike sign is, in view of the foregoing, untenable. It is true that such ions are adsorbed, but usually only by an equivalent exchange. A diminution in the potential could then, from this point of view, only result when the ingoing ion is less dissociated by the complex than the outgoing ion. It would be difficult to explain the flocculation of the Ca-saturated colloid by a sodium salt. Kruyt, who admits the equivalence of exchange, seems to forget the roll of the H ions already present in the As_2S_3 -complex, which he discusses when he says, "For the removal of the electric charge originally present, there is required, of course, a definite amount of positive electricity, or, in other words, a definite number of ions" and "that equivalent amounts of ions of different valence must be adsorbed in order to produce the same lowering of the interfacial potential" (11, p. 74). When the added electrolyte has the same cation

as the colloid there is no exchange and usually, therefore, no adsorption, yet flocculation will take place just as readily. A neutralization of the charge by adsorption would, in this case, be conceivable only if the added electrolyte suppressed the dissociation of the colloidal complex. But since the very opposite effect has been established, it is obvious that this theory cannot be accepted.

The net results of the foregoing may be summarized as follows:

The stability of the colloid depends upon the degree of dissociation of the complex, which in turn depends upon the nature of the ions of opposite sign of charge. The addition of an electrolyte results (a) in the formation, by exchange, of a new complex which may differ in stability from that of the original; (b) in a diminution in the activity, i.e., in the self-potential of all ions resulting; (c) in a higher degree of dissociation of the complex; (d) in a suppression in the P.D. between the micellar and intermicellar solutions according to the Donnan equation; and (e) in a suppression of the excess osmotic pressure within the micellar solution, resulting in a diminution in the osmotic hydration of the micelles.

How largely each of these and other, as yet unknown, changes contribute to the conversion of a stable sol into a coagulated mass is, at our present stage of knowledge, impossible to say. We know as yet too little about interfacial structure and interfacial forces. Interionic attraction, ionic association, molecular orientation, changes in dielectric constant, hydration, etc., all of which have been discussed in the foregoing, must be more fully understood before a successful solution of the problem is possible.

Hydration

It is not at all certain that the interfacial potential is directly responsible for the stability of a colloid, as already pointed out with reference to the work of Porter and Hedges (25). The charge of the particles must, however, be indirectly connected with the stability. Thus the osmotic hydration is proportional to the degree of dissociation of the complex, and hydration certainly is a stability factor. The great stability of the lyophile colloids, which do not flocculate until dehydrated at "salting out" concentrations, is well known. Whereas the emulsoid or lyophile colloids, like agar and gelatin, adsorb water by molecular attraction, the suspensoid type of colloid, like the soil minerals, do not attract water in this manner; but like the lyophile colloids, the suspensoid colloids imbibe water osmotically, the imbibition being proportional, as has been shown, to the degree of dissociation and therefore related to the charge. Hence soil colloids having a low exchange capacity imbibe less water and are, as will be shown, less stable than the colloids with a high exchange capacity. Similarly a given soil colloid saturated with NaOH will imbibe more water and be more stable than when saturated with $\text{Ca}(\text{OH})_2$. When dehydrated with alcohol, the soil colloids remain stable, provided all free electrolytes have first been removed as in electrodialysis, but become in this condition extremely sensitive to flocculating agents.

Table 28 shows the action of NaCl and KCl on Na- and K-saturated Norfolk and Sharkey soil colloids, respectively, in aqueous and in 75 per cent (by volume) alcoholic suspensions.

Table 28 yields the following information:

1. The lower the exchange capacity and the lower therefore the number of cations dissociated, resulting in a lower osmotic imbibition, (Comp. table 14), the greater is the sensitivity of the colloid to the flocculating action of the electrolyte. Thus the Norfolk colloid is flocculated in concentrations about ten times lower than is required by the Sharkey colloid. 2. The lyotropic effect is very great, as shown by the influence of the different cations. Thus the colloid which is saturated with the more hydrated and more extensively dissociating Na ions and which carries therefore a thicker envelope of osmotically imbibed water is less sensitive to the action of electrolytes. Thus it requires about twice as much NaCl as KCl for complete

TABLE 28
Flocculation of Na- and K-saturated Norfolk and Sharkey soil colloids in aqueous and alcoholic suspensions by NaCl and KCl

Na-SHARKEY		K-SHARKEY		Na-NORFOLK		K-NORFOLK	
NaCl N	Flocculation	KCl N	Flocculation	NaCl N	Flocculation	KCl N	Flocculation
<i>Aqueous suspensions</i>							
0.07	0	0.025	0	0.003	0	0.002	0
0.08	xx	0.030	x	0.004	x	0.003	xxx
0.09	xxx	0.035	xx	0.005	xx	0.004	xxxx
0.10	xxxx	0.040	xxx	0.006	xxx
.....	0.045	xxxx	0.007	xxxx
<i>Alcoholic suspensions 75 per cent absolute by volume</i>							
0.0008	0	0.0004	0	0.0008	0	0.0002	0
0.0010	xxx	0.0005	x	0.0010	xx	0.0003	xxxx
0.0012	xxxx	0.0006	xx	0.0012	xxxx
.....	0.0008

Exchange capacity m.eq./gm.: Sharkey, 0.796; Norfolk, 0.207.

Silica/sesquioxide ratio: Sharkey, 3.18; Norfolk, 1.63.

flocculation of each of the aqueous suspensions. It will be noted that this explanation assigns the lyotropic effect to the cation in combination with the colloid rather than to the cation of the free salt. The ions of the free electrolyte can only exert a suppressing effect and this suppression is apparently alone governed by the concentration and valence according to the Donnan equation, as was found in the case of the nonadsorbable anions. 3. The addition of alcohol dehydrates the ions thus increasing their activity, that is, their self-potential. This leads to a diminution in the degree of dissociation and consequently also to a decrease in the osmotic imbibition, as shown in table 11. The result is a greater sensitivity of the colloids, approaching in this respect the Ca-saturated materials. The flocculating value is greatly reduced, being now around 0.001N or about that of the Ca-saturated colloid. It will also be noted that the great differences in sensitivity between the two colloids in aqueous suspensions tend to vanish in alcoholic suspensions.

It is very essential that a distinction be made between molecularly attracted water and osmotically imbibed water. It is evident that Kruyt, in his study of the lyotropic influence, fails, with others, to recognize the latter form of hydration when he states, "If it (the lyotropic influence) were merely a matter of hydration, one would expect the strongly hydrated lithium ion to be a better flocculating agent than the slightly hydrated potassium ion, whereas the reverse is true" (11, p. 236). In other words, we should expect the Li ion to compete more strongly for the water and remove the protecting water envelope from the micelles and thus render them more sensitive. This is actually what takes place in the salting-out process of emulsoid colloids. Here the concentration is so high that the ions have to compete for the water. The most strongly hydrated ions are therefore the most active salting-out agents. The same applies to the increase in the activity coefficient in strong solutions. In flocculation, on the other hand, the concentration is so low as to exclude any such competition for the water molecules. The Li ion is the weakest flocculating cation for the same reason that it is a strong salting-out ion. It is strongly hydrated and therefore highly dissociated by the complex, giving rise to the highest degree of osmotic hydration, thus increasing the stability of the micelles. When a lithium salt is added to the negative colloid there is an exchange of cations, and a complex partly saturated with this cation is formed which will possess a higher charge and a higher osmotic hydration. A higher concentration is therefore required to suppress this stability factor to its critical value.

The foregoing observations would seem to justify the conclusion that the osmotic hydration is a dominating factor of stability. This does not eliminate the influence of the charge, because a high charge means a high dissociation and a high hydration. But it makes unnecessary the very doubtful assumption of an electrical repulsion. A repelling force is not necessary to account for the stability. If we assume a "radius of adhesion" within which the attracting force (which certainly does exist) alone is effective, then it is obvious that the micelles will remain dispersed as long as their radius of hydration is greater than the radius of adhesion. Under this condition the Brownian movement is sufficient to account for the dispersion of the colloid. Rapid flocculation will begin when the two radii are equal. This will correspond to a certain "critical potential" which might very well vary, as has been shown, with the nature of the ions, that is, the radius of adhesions might, like the radius of hydration, be a variable factor.

The attracting force

The next question is, what is the nature of the attracting force? Flocculation can hardly be the result of cohesive forces between the molecules of the dispersed phase, because the particles apparently do not come into actual contact. In ageing of a coagulum and in drying, cohesive forces doubtless come into play but flocculation apparently depends upon a looser attachment caused probably by ionic interaction. A "relative displacement by induction

between the two members of the double layer so as to give it an electrical moment," as suggested by Porter and Hedges (25), would account for such an attachment. Several years ago the author offered a somewhat similar explanation. It was assumed that the ions of the outer layer mutually attract the inner layers of two particles thus establishing a linkage between them (15). This would be analogous to the sharing of electrons by atoms forming molecules, as when two H atoms or Cl atoms unite to form a molecule. Our conceptions of atomic structure and micellar structure have much in common. The atom, consisting of a positive nucleus surrounded by the negative electrons, presents an electrical double layer similar to that of the micelle. If the electrically neutral atoms can unite by sharing some of their electrical units, it is only logical to apply the same reasoning to the union of colloidal micelles.

FACTORS GOVERNING THE SIZE OF THE PARTICLES

Size and exchange capacity

Anderson and Mattson (1), who determined ultramicroscopically the particle size in a series of soil colloids, found, as in the case of several other properties, that the dispersibility of the materials varied according to their composition and exchange capacity. Thus the higher the ratio of silica to sesquioxides and the higher the capacity to adsorb and exchange bases, the greater the degree of dispersion. For the two colloids here studied the following figures are presented:

COLLOID	SiO ₂	EXCHANGE CAPACITY M.EQ./GM.	NUMBER OF PARTICLES PER GM.	AVERAGE RADIUS, $\mu\mu$
	Al ₂ O ₃ + Fe ₂ O ₃			
Sharkey	3.18	0.796	960×10^{12}	45.5
Norfolk	1.63	0.207	322×10^{12}	64.5

The values represent the dispersion brought about by the addition of NH₃ to the original colloids saturated with various cations and must not be mistaken for "primary particles." For the Na-OH-saturated Sharkey and Norfolk colloids the author estimated, by an indirect method, an average particle radius of 34 and 46 $\mu\mu$ respectively (18).

That a relationship exists between this systematic variation in the size of the particles and the previously discussed colloidal behavior becomes quite evident. Why then are the particles of the Norfolk group of soil colloids larger than those of the group to which the Sharkey belongs? And why are the Na-saturated particles smaller than the K- or NH₄-saturated?

Before we attempt to answer these questions it is necessary to distinguish between two kinds of particles of colloidal dimensions: (a) Particles formed by the mechanical disintegration of rocks consisting of crystalline rock fragments. Such particles are abundantly present in glacial clays but must exist to some extent in all soils. The size of these articles is the result of a mechanical

accident. (b) Particles formed by the interaction between the hydrous oxides of silicon, aluminum, and iron and the various bases all formed as a result of a chemical weathering of the different minerals. It is this material which possesses the power of base exchange in a high degree and makes up the bulk of the soil colloids here studied. These particles may be looked upon as synthetic aggregates being built up from the various component or constituent parts. The size of the latter particles cannot be accidental but must be the result of an equilibrium between two opposing forces. One of these would expend the free energy by the highest possible degree of aggregation whereas the other, if acting alone, would reduce the free energy content by a higher dispersion. The magnitude of the former would be increased by dispersion, that of the latter by aggregation. The resultant of the two opposing forces must lead to a particle size of such dimensions as to maintain the total free energy at a minimum.

In the first we recognize the force of cohesion represented by the stray fields or residual valences which attract and arrange the molecules in the form of a crystal lattice. As opposed to this force, we must consider the electrokinetic and osmotic forces arising from a dissociation of the complex and resulting in a potential difference, in an osmotic hydration, and probably also in a lowering of the interfacial tension.

Ion density and dispersion

Now it can readily be visualized how these forces operate in the formation of a particle of maximum stability. Let us take a concrete example and consider the Sharkey and Norfolk soil colloids for which data are available. In a Norfolk particle of radius 64.5μ there are about 390,000 exchangeable univalent cations. In a Sharkey particle of the same radius the number of cations would be $.796/.207 \times 390,000 = 1,500,000$. Now if 390,000 cations, at a certain degree of dissociation, are sufficient to overcome the cohesive force, then, is obvious that a Sharkey particle containing nearly four times as many cations would not be stable. The ion density, at the same degree of dissociation, would be too great for stability, thus bringing about a greater dispersion. The Sharkey particle, according to the foregoing data, attains stability at a radius of 45.5μ and a cation content of 505,000. This would, at the same degree of dissociation, still give a considerably higher ion density to the Sharkey than to the Norfolk particle since the surface of a single particle varies as the square of the radius. But according to table 14 (part I), the Norfolk colloid is relatively more dissociated than the Sharkey. It is also obvious that the cohesive force may differ in the two materials. It appears quite evident, however, that the size of the particles is closely related to the number of exchangeable cations just as the osmotic imbibition was found to be so related.

The dispersing action of the dissociated ions may be seen to operate in the following way. The dissociated ions exert an outward pull on the surface layer of ions of unlike sign, thus tending to pull the particle apart. All capillary spaces that might be formed or that are already existing will be filled with liquid

into which the dissociated ions diffuse and establish an osmotic pressure, in excess of that of the outside solution, which will have a tendency to wedge the particles apart. As long as these forces are great enough to overcome the force of cohesion the material will continue to disperse. When once separated the particles are surrounded and protected by a layer of water, the water of osmotic hydration. The dispersing action of the dissociated ions will decrease with the ion density. If the degree of dissociation remained constant the ion density would vary inversely as the surface. For a given quantity of material, the surface varies inversely as the radius, hence the dispersing action of the ions must decrease with a decrease in the radius.

Distinction between aggregation and flocculation

This process of breaking up of coherent aggregates, desaggregation—is independent of the phenomenon of deflocculation. Desaggregation is opposed by the cohesive forces, whereas the forces holding the micelles together in the flocculated condition may be due to an interionic attraction, as has been explained. It is certain that cohesive forces do not here come into play. This can be demonstrated by the following simple experiment. If a piece of bentonite is placed in a NaCl solution of a concentration higher than that required to flocculate, e.g., 0.02 *N*, it will swell considerably but the individual micelles will not diffuse into the supernatant liquid but leave a sharp boundary between the gel and the liquid. If the salt concentration is 0.005 *N* or even 0.01 *N*, the micelles will diffuse into the liquid forming a suspension of decreasing density. In both cases there is a desaggregation but a deflocculation only in the latter. In both cases the cohesive force is overcome by the electrical and osmotic forces of the dissociated ions and the particles are forced apart a distance equal to twice the thickness of the layer of osmotic hydration. At this distance the forces of flocculation come into play in the stronger solution, linking the micelles together. In the weaker solution the micelles are not subject to any mutual attraction and are therefore carried farther apart by the Brownian movement.

Cohesion and aggregation

We have considered the forces leading to a dispersion of larger aggregates into smaller. Let us now consider the conditions which must lead to a condensation or aggregation. Why do the molecules or small units of associated molecules formed in the process of hydrolysis condense into aggregates of such dimensions as we find in the Sharkey and Norfolk soil colloids? To answer this question let us consider a Sharkey and a Norfolk aggregate each so small as to contain only one exchangeable cation. From analytical data (16, 17) the following figures have been calculated, assuming a sp. gr. of 2.65 and a value of 6.06×10^{23} for the Avogadro number:

COLLOID	RADIUS OF PARTICLE	NUMBER OF EXCHANGEABLE IONS	NUMBER OF MOLECULES OF SiO ₂	NUMBER OF MOLECULES OF Al ₂ O ₃ + Fe ₂ O ₃
	$\mu\mu$			
Sharkey	45.5	505,000	5,440,000	1,710,000
	0.57	1	10.8	3.4
Norfolk	64.5	390,000	12,330,000	7,500,000
	0.88	1	31.4	19.2

Two such particles would have a diameter of 1.14 and 1.76 $\mu\mu$, respectively. With a maximum of only one electronic charge, assuming complete dissociation, the particles would be very large as compared to the common ions. Their surface would be about 8 and 19 times as great as that of an ordinary ion, to which we may assign a diameter of 0.4 $\mu\mu$. As ions, they would therefore be very sluggish, showing a slow electrical migration. In this respect they would act like the large complex ions which show only a slow movement in the electric field as compared to the common ions and to the larger colloidal particles which possess a higher ion density.

When this line of reasoning is applied it becomes evident that these particles, although very small, are too large, as ions, to exist alone. The osmotic hydration in the case of a particle dissociating a single ion would, of course, be negligible. The force of molecular cohesion would assume full sway, resulting in a condensation until arrested by an increment of sufficient magnitude in the ion density. Since the number of ions in a particle increases with the cube of the radius, while the surface increases with the square, the increment in ion density would be proportional to the increase in the radius.

It is very improbable that a particle built up in this way would be as large as the Sharkey and Norfolk soil colloid particles. These colloids have been subjected to repeated drying and wetting. Whereas the lyophile colloids, like gelatin and agar, in which the cohesive force is lowered by a high degree of hydration by molecular attraction, may be completely reversible, the reversibility of the suspensoid type of colloids, like those of the soil, is certainly incomplete after the material has once been dried. This was illustrated in table 14, where it was shown that the oven-dried, K-saturated Sharkey colloid would no longer swell. From the great number of undissociated ions present in the soil colloid particles it is evident that if the cohesive force could once be overcome, particles of a much smaller size could be stabilized by an increase in dissociation. An artificial alumino-silicate, having an exchange capacity of the same order of magnitude as that of the natural colloids and which in the Na-saturated condition forms a perfectly clear sol, may be prepared. When dried it is no longer redispersible to the same degree. The condensation-born particle is therefore smaller than the dispersion-born particle. But since there must always be an equilibrium between the two opposing forces, the degree of dissociation in the case of a given material must be greater the smaller the particles.

The relation of the two opposing forces to the radius of the particles is shown graphically in figure 5. The free energy due to molecular attraction, that is to the force of cohesion, increases with the total surface of the dispersed phase and is therefore inversely proportional to the radius of the particles. If the quantity, cohesive force \times surface, is plotted against the radius a curve similar to C and C_1 is obtained. The ion density must vary inversely with the total surface and is therefore directly proportional to the radius as long as the number of ions dissociated remains constant. If the quantity, $\frac{\text{number of ions}}{\text{surface}}$, is

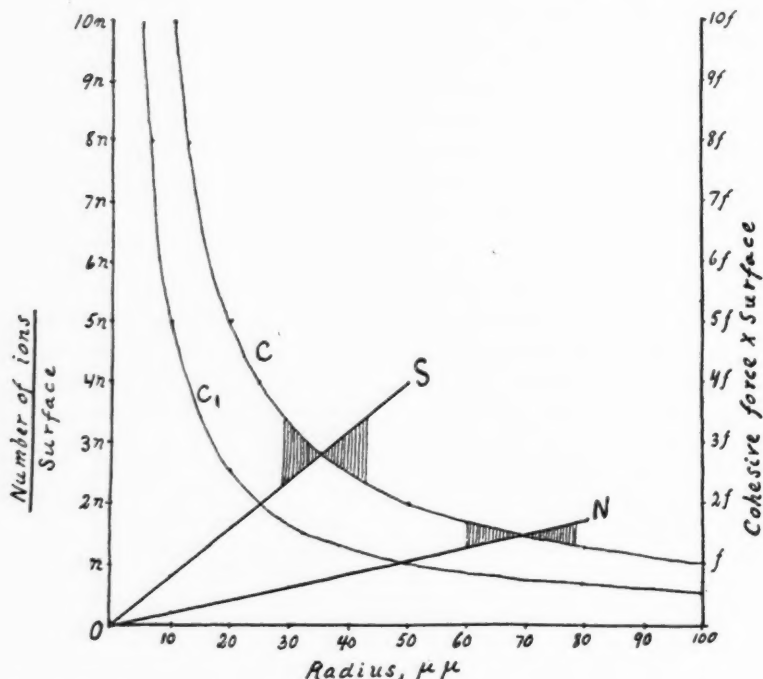


FIG. 5. THE RELATION BETWEEN THE SIZE OF THE PARTICLES, THE SURFACE DENSITY OF IONS, AND THE FORCE OF COHESION

plotted against the radius, curves similar to OS and ON are obtained. The slope of these curves will depend upon the number of ions dissociated. If the degree of dissociation of the Sharkey and Norfolk soil colloids were the same, then the ion density on particles of the same radius would be in the ratio of 0.796:0.207. The slopes of the curves OS and ON are in this ratio. It will be seen that these curves intersect C at points corresponding to radii of the relative values of 36 and 68 respectively. This ratio is a little wider than the ratio

of the radii found for the colloids and would indicate a higher dissociation of the Norfolk. The radius corresponding to the point of intersection represents the radius of the most stable particles. An increase or a decrease in size would make the particles increasingly unstable, as indicated by the shaded sections.

In the case of the same colloid saturated with different bases, the cations of which remain dissociated in various degrees, we obtain the same relationship. Thus let the curve OS represent the Na-saturated Sharkey and the curve ON the same colloid saturated with a cation only about one-fourth as highly dissociated. The ratio of the radii would again be as represented in the figure.

The curve C_1 is plotted to show the influence of a decrease in the force of cohesion, as in the case of the lyophile colloids or that of a lyophile colloid acting as a protecting agent in the formation of a lyophobe colloid. The molecules of a lyophile colloid have a strong attraction for water and a correspondingly weak attraction for one another. Equilibrium between the two forces will therefore be attained at a lower ion density and a correspondingly lower radius, as shown by the points of intersection with curve C_1 .

Flocculation and charge

We are now in a position, perhaps, to account for the fact that the highly dispersed bentonite shows an increase in the speed of migration in the first stage of flocculation. We have seen how an aggregation of smaller particles into larger, results in an increase in the ion density. This must necessarily result in a corresponding increase in the cataphoretic potential. Although flocculation does not, according to the views here presented, decrease the actual surface of the particles, it will, insofar as the ionic atmospheres overlap one another, cause a crowding and a lateral displacement of the ions. This will increase the mean distance from the inner layer, resulting in an increase in the cataphoretic potential. But since flocculation is conditioned by the presence of a certain quantity of free electrolyte and since all free electrolytes exert a suppressing influence, the aforementioned effect is generally more than balanced by the suppression of the ion activity. Only in the case of a highly dissociated and highly dispersed material like the Na-saturated bentonite does the former effect seem to outstrip the latter. This must be due to the great number of micelles present in each cluster when this suspension is flocculated. Another sample of bentonite from a South Dakota deposit, saturated chiefly with Ca and Mg and only very slightly dispersible even after saturation with Na, did not show the above anomaly (16).

In closing, another flocculation anomaly, unexplainable on the basis of the generally accepted theory, will be given. The Aragon soil colloid which was found to be isoelectric in old, CO_2 -contaminated water did not flocculate in this condition, only a slow sedimentation, due to the large size of the particles, being observed. The addition of 2 milliequivalents $\text{Ca}(\text{OH})_2$ per liter resulted in rapid flocculation although the particles migrated now toward the anode with a speed of 0.8μ per second. In the isoelectric condition the particles can have no

ion atmosphere. The addition of $\text{Ca}(\text{OH})_2$ resulted in a negative charge, indicating a cation atmosphere. Thus it appears very much as if flocculation, which must be looked upon as a clustering of the micelles and not as a molecular union of the particles, is conditioned by the existence of an ion atmosphere. This would be in harmony with the theory of ion linkage, according to which the particles are linked together by the ions of opposite charge. Where there is no ion atmosphere there is no connecting links and no flocculation. Aggregation by molecular attraction is then alone possible. But an ion atmosphere is alone not sufficient to attract and link the micelles together. Some stability factor such as hydration and possibly also molecular orientation must be broken down. This is accomplished by the free electrolyte, the presence of which appears to be essential to the process of flocculation. Whereas the forces of molecular aggregation and desaggregation, which determine the particle size, operate under all conditions, flocculation is the result of a more or less abrupt change in the stability of the micelles, brought about at a critical concentration of free electrolytes. Thus we find all sizes of particles in stable conditions depending on the nature of the material and the ions in combination.

SUMMARY

Flocculation and cataphoresis in solutions of various electrolytes and mixtures of electrolytes have been studied in the case of bentonite and soil colloids having different silica/sesquioxide ratios and different exchange capacities.

The charge and stability of the colloid depend upon the degree of dissociation of the exchangeable ions, which varies with their valence, hydration, potential, etc.

The theory which connects the charge and the stability with an adsorption of the ions of the added electrolyte is not supported. Thus the ions of the same sign as that of the colloid influence the stability according to the valence rule as established for the suppression of the swelling, although the ions are negatively adsorbed. The same must be true of the ions of opposite sign of charge, but here the valence effect is greatly exaggerated because of the formation of a new complex by exchange with each change of ion. The ions of opposite sign always enter into an exchange reaction with the ions present in the complex. If the ingoing ion is more highly dissociated by the complex than the ion originally present, then the charge, the osmotic hydration, and the dispersion tend to increase; if less dissociated, these values will all decrease and the sensitivity to electrolytes will increase. The free electrolyte exerts only a suppressing effect, and this in accordance with the valence and concentration of the ions. But the suppression of the charge and of the stability by free electrolytes is not, in the case of the common ions, due to an adsorption of the ions of opposite sign of charge except insofar as an exchange takes place; nor is it due to a suppression of the dissociation except insofar as the ingoing ion is less dissociated by the complex. On the contrary, the addition of electrolytes always increases the dissociation of the colloidal complex. This is identical with the salt effect on

weak electrolytes and is explained by a decrease in the activity of the ions according to the theory of interionic attraction. The abnormally large observed increase in dissociation is accounted for by the highly multivalent character of the particles. A linear relation between the dissociation and the square root of the ionic strength was brought out. An examination of the data supplied by Loeb reveals a similar relationship in the case of the proteins.

The quantity and quality of the exchangeable ions determine, through their various degrees of dissociation, the colloidal behaviors such as charge, osmotic hydration sensitivity to electrolytes, and dispersibility, that is, the size of the particles as determined by molecular aggregation.

The particle size is seen as the result of a balance between the force of cohesion on the one side and the force of dispersion, as determined by the ion density, on the other. This will result in a size of maximum stability for each colloid, depending on the nature and quantity of exchangeable ions. The relation between the observed size of particles in different colloids and the exchange capacity is brought out in a graph in which the ionic density and cohesive energy is plotted against the radius.

A distinction is made between aggregation and flocculation. Aggregation (and this also applies to disaggregation), which determines the size of the particles, is a continuous process which operates under all conditions. Flocculation is a discontinuous process in which the micelles (not the particles) are linked together, apparently by an electrostatic attraction. It seems to depend upon the existence of an ion atmosphere but takes place only in the presence of free electrolyte, the amount of which depends more on the degree of hydration than on the magnitude of the charge. There is no one critical potential at which soil colloids flocculate. Flocculation is often observed at a higher cataphoretic potential than that of the original stable suspension.

The osmotic hydration is the most potent stability factor. Thus the concentration required to flocculate a Na-saturated colloid having a high exchange capacity, which means a high osmotic hydration, is very much higher than in the case of a Na-saturated colloid with a low exchange capacity, although the initial charge may be the same. This difference in stability vanishes if the colloids are dehydrated with alcohol, in which condition they become extremely sensitive to electrolytes.

Although dispersion and particle size are held to be closely related to the charge, it is very doubtful whether the stability of the micelles depends upon a mutual repulsion. The osmotic hydration of the micelles is sufficient to account for the stability of a suspension. Since this hydration depends upon the number of dissociated ions, the connection between a high charge and the stability of a suspension, which is often observed but which is not general, is seen as an indirect effect.

REFERENCES

- (1) ANDERSON, M. S., AND MATTSON, SANTE 1926 The properties of the colloidal soil material. U. S. Dept. Agr. Bul. 1452.
- (1a) BRÖNSTED, J. N. 1921 Influence of salts on chemical equilibria in solutions. *Jour. Chem. Soc.*, (London) 119: 574-592.
- (2) BRÖNSTED, J. N., AND LA MER, V. K. 1924 The activity coefficient of ions in very dilute solutions. *Jour. Amer. Chem. Soc.* 24: 555-573.
- (3) BURTON, E. F. 1909 The action of electrolytes on copper colloidal solutions. *Phil. Mag. and Jour. Sci.* (6) 17: 583-597.
- (4) CLARK, W. M. 1928 The Determination of Hydrogen Ions, ed. 3, chap. 25, p. 489-512, Baltimore.
- (5) DEBEY, P., AND HÜCKEL, E. 1923 Zur Theorie der Elektrolyte. *Phys. Ztschr.* 24: 185-206.
- (6) DEBEY, P., AND HÜCKEL, E. 1924 Bemerkungen zu einem Satze über die kataphore-tische Wanderungsgeschwindigkeit suspendierter Teilchen. *Phys. Ztschr.* 25: 49.
- (7) EHRENBURG, PAUL 1918 Die Bodenkolloide, ed. 2, p. 303, Leipzig.
- (8) GEDROIZ, K. K. 1924 The ultra mechanical composition of soils and its dependence on the nature of the cations present in the soil in adsorbed condition. *Zhur. Opitn. Agron.* 22: 29.
- (9) GIVEN, G. 1915 Dissertation Göttingen, in Die Bodenkolloide, by P. Ehrenberg, ed. 2, p. 568. Leipzig, 1918.
- (10) HARDY, W. B. 1900 Eine vorläufige Untersuchung der Bedingungen, welche die Stabilität von nicht umkehrbaren Hydrosolen bestimmen. *Ztschr. Phys. Chem.* 33: 385-400.
- (11) KRUYT, H. R. 1927 Colloids. New York.
- (12) KRUYT, H. R., AND VAN DER WILLIGEN, P. D. 1927 Kataphorese und Ladung. *Ztschr. phys. Chem.* 130: 170-176.
- (13) LEWIS, G. N., AND RANDALL, M. 1921 The activity coefficient of strong electrolytes. *Jour. Amer. Chem. Soc.* 43: 1112-1154.
- (14) LOEB, JACQUES 1924 Proteins and the Theory of Colloidal Behavior, ed. 2. New York.
- (15) MATTSON, SANTE 1922 Die Beziehungen zwischen Ausflockung, Adsorption, und teilchenladung mit besonderer Berücksichtigung der Hydroxylionen. *Kolloid-chem. Beihefte* 14: 227-316.
- (16) MATTSON, SANTE 1926 The relation between the electrokinetic behavior and the base exchange capacity of soil colloids. *Jour. Amer. Soc. Agron.* 18: 458-470, 510-512.
- (17) MATTSON, SANTE 1926 Electrodialysis of the colloidal soil material and the exchangeable bases. *Jour. Agr. Res.* 33: 553-567.
- (18) MATTSON, SANTE 1927 The influence of the exchangeable bases on the colloidal behavior of soil materials. *Proc. First Internat. Congr. Soil Sci.* 2: 185-198.
- (19) MATTSON, SANTE 1927 Anionic and cationic adsorption by soil colloidal materials of varying $\text{SiO}_2/\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3$ ratio. *Proc. First Internat. Congr. Soil Sci.* 2: 199-211.
- (20) MATTSON, SANTE 1928 The electrokinetic and chemical behavior of the aluminosilicates. *Soil Sci.* 25: 289-311.
- (21) MATTSON, SANTE 1928 Cataphoresis and the electrical neutralization of colloidal material. *Jour. Phys. Chem.* 32: 1532-1552.
- (22) MATTSON, SANTE 1928 The action of neutral salts on acid soils with reference to aluminum and iron. *Soil Sci.* 25: 345-250.
- (23) MÜLLER, HANS 1928 Sur Theorie der elektrischen Ladung und der Koagulation der Kolloide. *Kolloidchem. Beihefte.* 26: 257-311.

- (24) NOYES, A. A. The inter-ionic attraction theory of ionized solutes. II. Testing of the theory with experimental data. *Jour. Amer. Chem. Soc.* 34: 1098-1116.
- (25) PORTER, A. W., AND HEDGES, J. J. 1922 The law of distribution of particles in colloidal suspensions with special reference to Perrins investigations. *Phil. Mag. and Jour. Sci.* (6) 44: 641-651.
- (26) POWIS, FRANK 1914 Die Beziehung zwischen der Beständigkeit einer Ölemulsion und der Potentialdifferenz an der Öl-Wasser-grenzfläche und die Koagulation kolloider Suspensionen. *Ztschr. Phys. Chem.* 89: 186-212.
- (27) TAYLOR, H. S. 1925 A Treatise on Physical Chemistry, vol. 2, p. 765. New York.
- (28) TUORILA, PAULI 1928 Über Beziehungen zwischen Koagulation, elektrokinetischen Wanderungsgeschwindigkeiten, Ionenhydratation und chemischer Beeinflussung. *Kolloidchem. Beihefte* 27: 44-188.
- (29) The theory of strong electrolytes. A general discussion. 1927 *Trans. Faraday Soc.* 23: 333-542.